BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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Contents of Volume LXIII

No. 1. July, 1922.

Newman, H. H. *Normal Versus Subnormal Development in Patiria miniata. A Caution to Laboratory Embryologists* 1

Breitenbecher, J. K. *Somatic Mutations and Elytral Mosaics of Bruchus* 10

Plath, O. E. *Notes on Psithyrus, with Records of Two New American Hosts* 23

Humphrey, R. R. *The Multiple Testis in Urodeles* 45

Glaser, Otto. *The Hydrolysis of Higher Fats in Egg-secretion* 68

No. 2. August, 1922.

Springer, Mary G. *The Effect upon Developing Eggs of Extracts of Embryos of the Same Species* 75

Danchakoff, Vera, and Seidlein, S. M. *Digestive Activity of Mesenchyme and its Derivatives* 97

Sumner, Francis B., McDaniel, Mary E., and Huestis, Ralph R. *A Study of Influence which may Affect the Sex-Ratio of the Deer Mouse* 123

No. 3. September, 1922.

Jones, D. F. *Selective Fertilization and the Rate of Pollen-tube Growth* 169

Glaser, Otto. *The Temporary Concentration of Sea-salts About Arbacia Eggs* 175

Bowen, Robert H. *Notes on the Occurrence of Abnormal Mitoses in Spermatogenesis* 184

Mast, S. O., and Gover, Mary. *Relation Between Intensity of Light and Rate of Locomotion in Phacus pleuronectes and Euglena gracilis and its Bearing on Orientation* 203

Huxley, J. S. *Dedifferentiation in Echinus Larvæ, and its Relation to Metamorphosis* 210

No. 4. October, 1922.

Crozier, W. J. *"Reversal of Inhibition" by Atropine in Caterpillars* 239
Snyder, L. H., and Crozier, W. J. *Notes on Some Problems of Adaptation: 9. Ctenidial Variation in Chiton*.... 246
Metz, C. W. *Chromosome Studies on the Diptera. IV. Incomplete Synopsis of Chromosomes in Dasyllis grossa Fabr.* 253
Sampson, M. M. *Iso-agglutination and Hetero-agglutination of Spermatozoa* 267
Moore, C. R. *On the Physiological Properties of the Gonads as Controllers of Somatic and Psychical Characteristics, V.* 285

No. 5. November, 1922.

Hyman, L. H., and Bellamy, A. W. *Studies on the Correlation Between Metabolic Gradients, Electrical Gradients, and Galvanotaxis. I*..... 313
Richards, A. *The Acceleration of the Rate of Cell Division.* 348

No. 6. December, 1922.

Metz, C. W. *Association of Homologous Chromosomes in Tetraploid Cells of Diptera*..... 369
Parker, G. H. *The Geotropism of the Sea-urchin Centrechinus* 374
Just, E. E. *Initiation of Development in the Egg of Arbacia, I. Effect of Hypertonic Sea-water in Producing Membrane Separation, Cleavage, and Top-swimming Plutei* 384
Just, E. E. *Initiation of Development in the Egg of Arbacia, II. Fertilization of Eggs in Various Stages of Artificially Induced Mitosis*..... 401
Just, E. E. *Initiation of Development in the Egg of Arbacia, III. The Effect of Arbacia Blood on the Fertilization Reaction*..... 411

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CONTENTS

		PAGE
NEWMAN, H. H.	*Normal Versus Subnormal Development in Patiria miniata. A Caution to Laboratory Embryologists*	1
BREITENBECHER, J. K.	*Somatic Mutations and Elytral Mosaics of Bruchus*	10
PLATH, O. E.	*Notes on Psithyrus, with Records of Two New American Hosts*	23
HUMPHREY, R. R.	*The Multiple Testis in Urodeles*	45
GLASER, OTTO.	*The Hydrolysis of Higher Fats in Egg-secretion*	68

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BIOLOGICAL BULLETIN

NORMAL VERSUS SUBNORMAL DEVELOPMENT IN PATIRIA MINIATA. A CAUTION TO LABORATORY EMBRYOLOGISTS.

H. H. NEWMAN.

(From the Hopkins Marine Station of Leland Stanford University and the Hull Zoölogical Laboratory of the University of Chicago.)

(TWENTY-SIX FIGURES.)

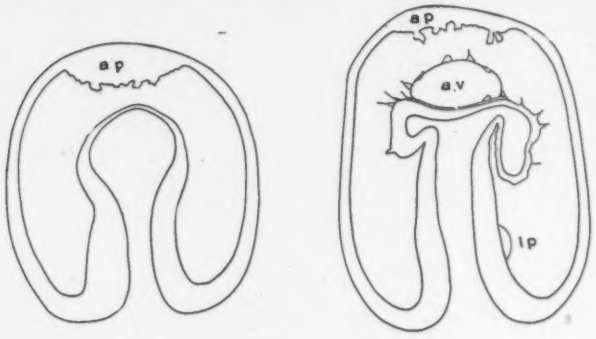
Years of experience in rearing marine organisms in finger-bowls of sea water have made the present writer somewhat skeptical as to the validity of life histories worked out under these conditions. The average morphologist who is unfamiliar with the modern refinements of experimental physiology or of ecology scarcely realizes how abnormal as the environment of a marine organism is a bowl of stale and stagnant sea water kept at room temperature and in reach of direct sunlight. Such an organism is adjusted in all of its physiology to the open sea with its uniform temperature, its delicately balanced oxygen content and hydrogen-ion concentration, and its opportunities for selecting light and shade of optimum intensities.

In view of these discrepancies between the natural and the artificial environment it would seem remarkable that finger-bowl cultures ever could approximate normal development. Some organisms, however, are hardy and tolerant of suboptimal conditions and develop in finger-bowls with only slight departures from the normal. Very many marine organisms are, on the other hand, extremely sensitive to suboptimal environmental conditions and show the effects of the finger-bowl environment all too plainly.

A conspicuous example of an organism highly sensitive to suboptimal environmental conditions is the egg of the California starfish, *Patiria miniata*, with which the writer (Newman, '21 *a*, '21 *b*) has dealt at some length, calling attention to the numerous anom-

A. P.

alies exhibited by the larvæ of this species, such as twin gastrulæ and *Bipennariæ* and numerous inhibited types. One would scarcely choose such a species as a favorable object for the study of normal development; yet this very thing has been done by Heath, who in 1917 published an account of the early development of this species.



1 *2*

FIG. 1. A gastrula stage, which is obviously subnormal and is to be compared with a normal gastrula such as is shown in FIG. 17. (After Heath.)

FIG. 2. A larva in the stage of cutting off the hydroenterocœl pouches. (After Heath.) *a. p.*, apical plate; *a. v.*, anterior vesicle; *l. p.*, left posterior enterocœle pouch.

Heath's account was, when it first appeared, very interesting to the present writer because of the rather startling discovery of several characters quite unknown for echinoderm larvæ, but suggestive of enteropneustan conditions. A brief account of Heath's findings must be given in order that the reader may appreciate the present contention.

In the plankton of Monterey Bay a small number of gastrulæ were found which seemed to Heath to combine in a unique way the characters of Echinodermata and of Enteropneusta. Various starfishes were artificially fertilized and reared far enough to show that the first captured plankton specimens were larvæ of *Patiria miniata*.

The artificially fertilized material was reared in finger-bowls and it was found that "the segmentation and early blastula stages do not exhibit any noteworthy features, but beyond this point certain structures arise that have no known counterpart among starfishes. The first of these unique organs is the *apical plate*. In the blastula

stage the cells about the animal pole commence to elongate and, in the gastrula, form a thickened area, more or less lens-shaped in form, having approximately one half the diameter of the transverse axis of the embryo. As indicated in the drawings (Figs. 1, 2), its center is exactly opposite to the blastopore and therefore is strictly apical." Heath notes later that "all of the cells of the apical plate are packed with granules, evidently yolk."

That Heath was entirely incorrect in his interpretation of the structure called by him the "apical plate" is easily shown. The

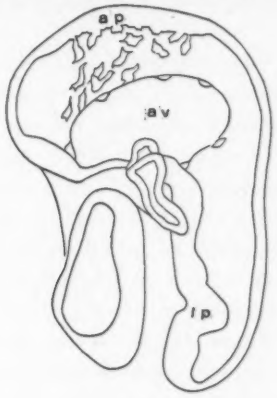


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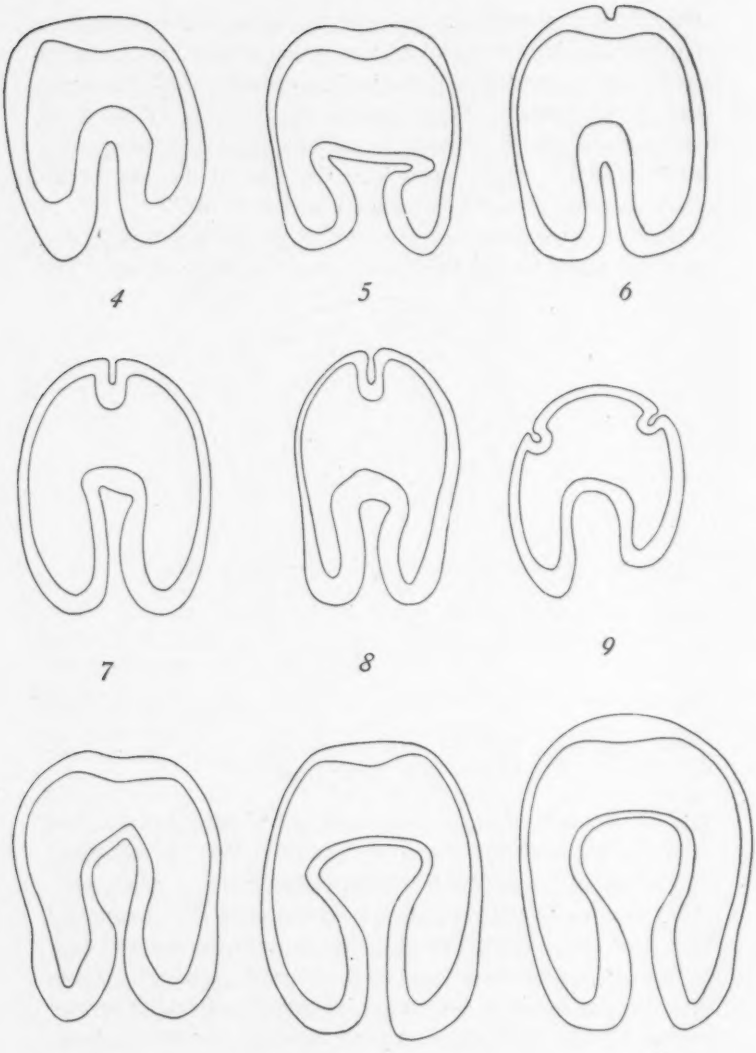
FIG. 3. A young *Bipennaria; a.p.*, "apical plate"; *a.v.*, "anterior vesicle"; *l.p.*, left posterior enterocœl. (After Heath.)

fact that the cells of this structure are packed with yolk granules should have given him a clue to the real condition; for yolk granules belong to the vegetal pole, not to the animal or apical pole. What Heath saw and accurately described were almost certainly subnormal or inhibited larvæ resulting from parthenogenetic eggs or from eggs inhibited by poor environmental conditions. These never develop normally, but always lag behind and exhibit various anomalous structures. Figures 4–12, inclusive, are camera drawings of a typical series of gastrulæ derived from parthenogenetic eggs of *Patiria,* reared by the writer in finger-bowls in the Pacific Grove Laboratory. These larvæ are all of the same age, about two days old, and are comparable with Heath's first figure (Fig.

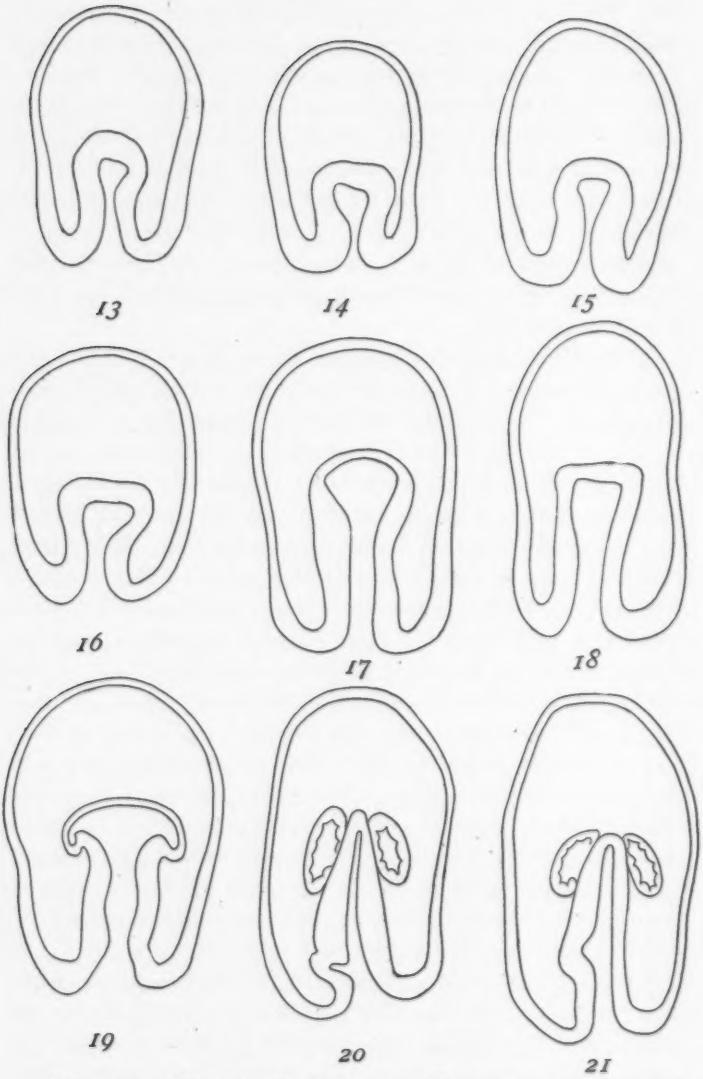


FIGS. 4–12. Camera lucida outlines of optical sections of a series of *subnormal* larvæ derived by spontaneous parthenogenesis from eggs of *Patiria miniata*. In these larvæ the ectoderm in the region opposite the blastopore is frequently thickened and also very frequently invaginates to form secondary archentera. These thickenings do not occur in normal larvæ as may be seen by comparing these figures with Figs. 13–21.

1). It will be noted that the so-called "apical plate" is shown in various forms, ranging from only a slight thickening to a small secondary invagination or even several invaginations. Figure 9 shows one larva with two small secondary archentera. The physiological interpretation of this process of twinning has been given in another paper and does not especially concern us here. Sufficient to say that the so-called "apical plate" is merely a secondary region of low metabolic activity or a secondary area of primitive endoderm, as is evidenced by the presence of yolk granules, and that such a region very frequently invaginates and forms an additional archenteron.

In the light of Heath's descriptions the above sounds like a fairly dogmatic statement and one that should not be made without complete proof. The proof of the above contention is to be found in a study of the truly normal development of the species. If one artificially fertilizes a good ripe lot of *Patiria* eggs, having only a single layer of eggs on the bottom of the dish, uses just enough and not too much sperm, washes out excess sperm and any fragments of ovary or testes after an hour or so, the following conditions will be noticed within about twenty-four hours. A large percentage of gastrulæ like those shown in the camera drawings (Figs. 13 and 14) are found swimming near the surface. These are the normal larvæ and such surface larvæ rarely show any trace of an apical thickening. Near the bottom of the vessel, however, one finds numerous larvæ of the types shown in Figs. 4–9, as well as much more inhibited types. The normal larvæ swimming near the surface are perfectly typical asteroid gastrulæ and are in no way aberrant. If these larvæ are skimmed from the surface and placed in water taken fresh from the open sea, they develop as shown in Figs. 15–23, showing no differences of consequence from larvæ of other asteroids studied side by side with them.

Returning once more to Heath's account, we note that he claims that "in fully 50 per cent. of the specimens in hand a few of the mesenchyme cells arising from the blind end of the archenteron, between the enterocele pouches, unite to form a small vesicle (Fig. 2, *a*, *v*). This usually occurs after the enterocele pouches are well differentiated, though not completely cut off." Heath seems to have seen such a thin-walled vesicle quite clearly, if one may judge



FIGS. 13–21. Camera lucida outlines of optical sections of normal larvæ derived from fertilized eggs and drawn off from the surface of the dish. These normal larvæ never show "apical plate" nor "anterior vesicles." FIGS. 13–15 drawn about 24 hours after fertilization. FIGS. 16–18 drawn at about 48 hours. FIG. 19 drawn at 30 hours. FIGS. 20 and 21 drawn at 72 hours.

by his figures. I must admit, however, that in normal material I never could see even the faintest suggestion of such a structure, while in subnormal material I occasionally observed what appeared to be a vesicle of some sort, but even in preserved material it never exhibited anything like complete continuity of outline. Heath describes this so-called "*anterior vesicle*" in great detail, dwelling on its supposed mesenchymal origin and its lack of communication with the archenteron. If, however, such a vesicle is absent in normal larvæ, discussion as to its exact structure loses significance.

A third peculiar structure is described by Heath: "a relatively

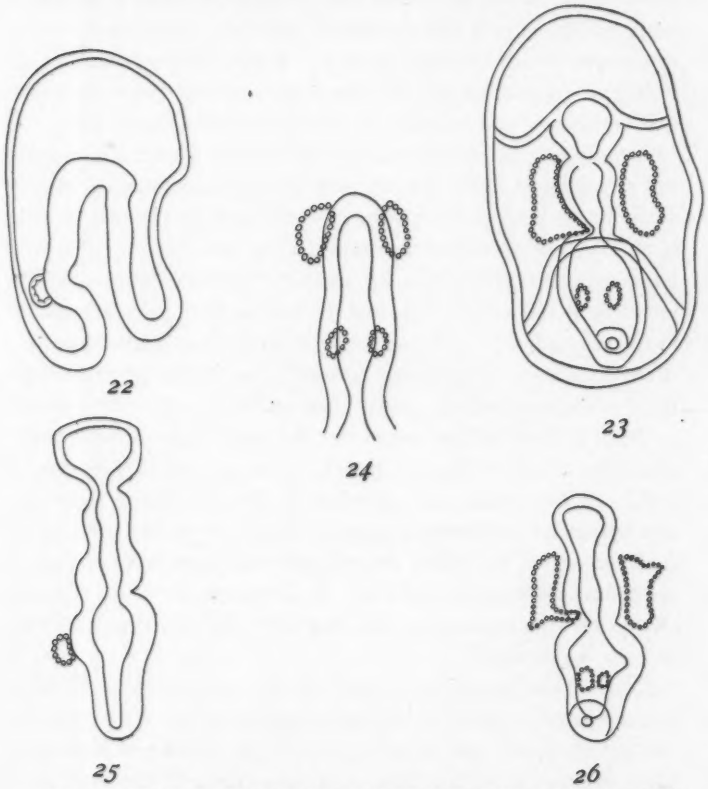


FIG. 22. Right lateral view of larva with single posterior enterocœle vesicle. FIGS. 23, 24, 26 show larvæ with paired posterior enterocœle vesicles. FIG. 25 shows in detail the alimentary tract of a larva with but one posterior enterocœle, on the left.

large vesicle (a posterior enterocœl pouch) present on the left side only." Gemmill had described for *Asterias rubens* a pair of posterior enterocœl pouches which seemed to play little part in the formation of the posterior cœloms, but merely produced mesenchyme. My own observations of the normal *Patiria* larva enable me to confirm Gemmill's findings. More frequently than not two small paired pouches, very thin-walled, appear at about the level of the future stomach. Sometimes only one such pouch appears. The pouch is never large nor thick-walled, as one might infer from Heath's account. The nearest semblance to his structure is seen in my Figs. 20 and 21, where the archenteron shows a unilateral outpouching. Since this condition is relatively rare, however, one can not lay much emphasis upon it. I was not interested in following up the fate of any of these structures, but Heath promises that an attempt will be made to determine their ultimate fate.

On the basis of his observations on *Patiria,* Heath allows himself to engage in rather far-reaching phylogenetic speculations and sums up his position as follows: "I am strongly inclined to look upon the anterior vesicle in *Patiria* as the homologue of the proboscis cœlom of *Balanoglossus,* while the posterior outgrowth corresponds to the trunk cœlom and the intermediate pairs of vesicles in the echinoderms, often with two hydropores in certain species, is the equivalent of the collar cœlom." In another place he says that his discovery of the apical plate in *Patiria* is a strong bit of evidence in favor of the theory that the apical plate of the trochophore larva and of the echinoderm larva are homologous structures. It may readily be seen that to the phylogenist these are speculations of considerable moment, inasmuch as they seem to be confirmatory of the rather current idea that there is more than a superficial resemblance between the Tornaria larva of *Balanoglossus* and the *Bipennaria* and *Auricularia* larvæ of the starfishes and sea-cucumbers.

I have been persuaded to publish this correction of Heath's account merely to avoid the strong probability of the error creeping into our textbooks and becoming part of our permanent literature. Many another error has been thus immortalized; for, once in a textbook, a statement, no matter how erroneous, seems to be passed from generation to generation.

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SOMATIC MUTATIONS AND ELYTRAL MOSAICS OF BRUCHUS.

J. K. BREITENBECHER,

Contribution from the Zoölogical Laboratory of the University of Oklahoma, Second Series, No. 10.

INTRODUCTION.

During the years 1918 to 1920 thirty-one unusual females were discovered in cultures of *Bruchus quadrimaculatus*.* These unusual types were normal insects except that the elytra were of different colors. Such striking differences as these constitute what the geneticists term mosaics, and because they appear in the elytra the author describes them as elytral mosaics. It is a noteworthy fact, proven by the experiments, that these mosaics are not transmitted; it is necessary, therefore, to assume that these peculiar differences must originate in somatic tissue. It will be evident from the experiment, described herein, that these mosaics manifest an order of dominance in agreement with that of mutations. The author, therefore, concludes that these elytral mosaics are caused by somatic mutations.

It is also a noteworthy fact that these mosaics were more abundantly manifested during the period in which the author was investigating the genetics of the first three mutants (red, black, and white) which he had discovered in the progeny of the so-called "four-spotted cowpea-weevil," *Bruchus quadrimaculatus* Fabr. In a previous paper (Breitenbecher, '21) the behavior of these mutants was described and a multiple allelomorph system demonstrated for the factors which involve the four-body and elytral colors as discovered previously for *Bruchus*. Here the author proved that the order of dominance for these four-color factors was red, black, white, and tan (the wild type). The females and males of the wild type have tan elytra and bodies, while the females

* Since this paper was sent to press, 17 new mosaics have been observed, making a total of 48 to date.

of the white mutant cultures have white elytra and bodies and the males have tan elytra but gray bodies. The black mutant stock shows a more pronounced dimorphism, since its females had black elytra and bodies, and its males tan elytra but grayish-black bodies. A lesser difference is seen in the red mutation, for its females had red elytra and bodies, while its males had tan elytra and reddish-gray bodies. It is evident from this description that every male had tan elytra. In conformity with an allelomorph series, the following formulæ were designated by the author: red (R R), black (R^bR^b), white (R^wR^w), and tan or wild (r r). These formulæ also indicate the order of dominance.

TABLE I.

THE DATA ON THE ORIGIN, APPEARANCE, AND OFFSPRING OF ELYTRAL MOSAICS IN BRUCHUS.

Mosaic No.	Date Discovered.	Found in a Pure Culture of	Mosaic Female Elytral Color		Bred to a Male Pure for	Offspring Summary.
			Left.	Right.		
1	1918 April 20	Black	Black	Red	Black	None
2	" May 18	"	"	"	"	"
3	" Aug. 18	Wild	Tan	Black	Wild	All wild
4	" Aug. 28	Black	Black	Red	Black	" black
5	" Sept. 21	"	Red	Black	"	None
6	" Sept. 30	Wild	Black	Tan	Wild	All wild
7	" Oct. 7	Black	"	Red	Black	None
8	" Oct. 15	"	"	"	"	All black
9	" Oct. 22	"	"	"	"	" "
10	" Nov. 7	"	"	"	"	" "
11	" Dec. 2	"	"	"	"	" "
12	" Dec. 12	"	Red	Black	"	" "
13	1919 Jan. 2	"	"	"	"	" "
14	" Jan. 5	Wild	Tan	"	Wild	All wild
15	" Jan. 12	Black	Black	Red	Black	" black
16	" Jan. 14	Wild	Tan	Black	Wild	None
17	" Jan. 16	Black	Red	Black	Black	"
18	" Jan. 17	Wild	Tan	"	Wild	"
19	" Jan. 21	Black	Black	Red	Black	All black
20	" Jan. 23	Wild	Tan	Black	Wild	None
21	" Jan. 23	Black	Red	"	Black	None
22	" Jan. 23	Black	Black	Red	"	All black
23	" Jan. 25	"	"	"	"	" "
24	" Jan. 27	"	"	"	"	" "
25	" Jan. 27	"	Red	Black	"	" "
26	" Feb. 12	"	"	"	"	" "
27	" Mar. 27	"	Black	Red	"	" "
28	" Apr. 12	"	Red	Black	"	" "
29	" July 16	Wild	White	Tan	Wild	All wild
30	" Dec. 9	"	Tan	Black	"	None
31	1920 Jan. 1	White	Black	White	White	All white

It is the purpose of this paper to describe the origin and genetic behavior of thirty-one elytral mosaic females which were discovered among thousands of normal insects examined during the progress of this work. The usual expectancy for homozygous cultures is that the elytra of the normal females will be red-red for the red mutant, black-black for the black one, white-white for the white mutant, and tan-tan for the wild type. But for these thirty-one cases, types of mosaic elytra have appeared, such as red-black, black-red, black-white, black-tan, tan-black, and white-tan. It is with these unusual types that this paper is concerned.

TABLE II.

Mosaic No.	F_1 Elytra Color.		F_2 Elytra Color.		F_3 Elytra Color.		F_4 Elytra Color.		Totals.
	♀♀	♂ Tan	♀♀	♂ Tan	♀♀	♂ Tan	♀♀	♂ Tan	
3	3 tan	3	5 tan	20	15 tan	20	21 tan	22	109
4	3 black	3	4 black	6	37 black	41	289 black	351	734
6	2 tan	2	3 tan	3	40 tan	24	120 tan	145	339
8	9 black	7	23 black	31	99 black	87	472 black	462	1,190
9	5 "	8	41 "	45	173 "	163	1,431 "	1,550	3,416
10	13 "	11	76 "	78	201 "	215	2,327 "	2,401	5,322
11	28 "	15	92 "	83	324 "	321	3,213 "	3,337	7,413
12	9 "	10	23 "	29	93 "	101	728 "	719	1,712
13	7 "	3	4 "	1					15
14	4 tan	6	13 tan	18	231 tan	239			511
15	9 black	2	10 black	8					29
19	9 "	19	83 "	91	514 black	565			1,275
22	2 "	2	21 "	28					53
23	8 "	6	139 "	174					324
24	10 "	8	3 "	7					28
25	3 "	4	97 "	93					197
26	42 "	47	317 "	323					729
27	5 "	6	98 "	91					200
28	5 "	8	202 "	224					439
29	15 tan	18	84 tan	89	327 tan	331			864
31	9 white	8	92 white	102	439 white	427			1,077

The Experiments.

The data and results from the breeding experiments which were carried on during the years 1918–1920 are summarized, as they bear upon the question of mosaics, in Tables I. and II. It seems easier to interpret these mosaics if one describes them in the order of their dominance (red, black, white, and tan, or the wild type).

It is evident from consulting Table I. that no mosaic has ever

appeared in any pure culture of red. This fact is significant because red is dominant to black, white, and tan, and if recessive mosaics through mutations occurred in the red stock, they could not be seen. It is of interest to observe that all thirty-one elytral mosaics were females; the reason is, the colors (red, black, and white) are somatically visible in the elytra of this sex, but, on the other hand, no elytral mosaic males were discovered because the elytra of the males of the four types are always tan. Therefore, if a mosaic was produced in a male, it could not be seen, because the characters involved are sex-limited traits.

Twenty-two of these mosaics, described in Table I., originated from homozygous, black cultures. Each of these mosaic females was alike in having a normal black body, except that one elytrum was always red. Of these, eight (Mosaics 5, 12, 13, 17, 21, 25, 26, and 28) had a red, left elytrum and a black, right one, while fourteen (Mosaics 1, 2, 4, 7, 8, 9, 10, 11, 15, 19, 22, 23, 24, and 27) had a black, left elytrum and a red, right one. In order to determine whether a factor mutation had occurred, each of these mosaic insects was mated to a homozygous, black male, and the number of offspring from each pair is given in Table II. The result is the same from every mating; red was not transmitted because all progeny were pure for black. No doubt the fact that red is the only dominant to black explains why the red elytrum was visible. The fact that the black mutant cultures are more prolific and, accordingly, give rise to a greater number of offspring might account for the appearance of more mosaics, unless it is assumed that the mutation from black to red occurs more frequently.

In order to illustrate the red-black, elytral, mosaic type, let us describe Mosaic 12 (Tables I. and II.) as an example. This insect appeared on December 12, 1918, from a pure, black culture. She had a red, left elytrum and a black, right one. She was mated to a pure, black male, because her body color was, likewise, homozygous for black; her progeny were every one pure for the black character. Among the 1,712 offspring no red ones were discovered; however, red was visible in the mother because it is a dominant color to black, which is its recessive allelomorph.

From the fourteen black-red, elytral mosaics let us select Mosaic 9. This animal emerged from a culture which was homozygous

for the black mutation on October 22, 1918. She possessed all the characters which were normal for a black female, except that her right elytrum was red. She was mated to a male with tan elytra, but pure for black, and this pair gave rise to 3,416 pure, black progeny. The same result is evident as before; red was not observed in any offspring, even though they were inbred for four generations; but red was visible, however, in the original parent because it is a dominant color to black.

It is next in order to consider the elytral mosaics which manifested themselves in a pure, white culture. The last mosaic (Mosaic 31, Tables I. and II.) described made its appearance on January 1, 1920, from a culture that was homozygous for the white mutant stock. Her body color and right elytrum were both normal for the white insect, but her left elytrum was black. She was bred to a male normal and pure for white. They produced 1,077 homozygous white descendants. This test indicates the same general behavior relative to dominance. Black is manifested here because it is a dominant color to white. It should be possible also to find among the progeny of a homozygous, white culture both red-white and white-red mosaics, but none were found, because it is almost impossible to keep this stock alive. This is not surprising, since only one mosaic was seen during the progress of this experiment.

Because the tan or wild type is recessive to any of the above body and elytral colors, their mosaics will next be considered. The following dominant mosaics are possible from this type: Tan-white, white-tan; black-tan, tan-black; red-tan, tan-red. Of these possible dominant mosaics three have appeared; the white-tan, tan-black, and black-tan. The others as yet have not occurred.

Thus far only one individual of the white-tan type has been discovered, Mosaic 29 (Tables I. and II.). On July 16, 1919, a remarkable elytral mosaic was found which was different from any of the others. This female had a white, left elytrum and a tan, right one; her body was normal, however, for the wild type. She originated from a wild culture, and when bred to a wild (tan) male produced 864 wild progeny. White was also visible in this elytral mosaic female because white is a somatic dominant color to tan.

This proves that white was not transmitted to any offspring; on the other hand, no tan-white insects have so far appeared.

Another kind of mosaic that was found frequently among the offspring from the wild cultures was an animal with one black elytrum. Six (Mosaics 3, 14, 16, 18, 20, and 30) of these females had tan, left elytra and black, right ones, while one (Mosaic 6) had a black, left elytrum and a tan, right one.

To illustrate the tan-black type, Mosaic 14 will be described. A mosaic female was observed after she had emerged with many other insects from a wild culture on January 5, 1919. This female had a normal, tan body and tan, left elytrum, while her right elytrum was black. When mated with a normal (tan) wild male, her offspring produced through three generations 511 tan or wild descendants. This type of a mosaic proves that black was not transmitted, because it did not appear in any offspring; again black appeared in this insect because black is a dominant to tan, its recessive allelomorph.

Of the black-tan, Mosaic 6 is the only one which was observed. She was found in a culture from wild parents on September 30, 1918. Her body and right elytrum were both tan, but her left elytrum was black. She was mated to a wild male and produced 339 homozygous, wild progeny. The result shows that the black visible in this mosaic was not transmitted because it is a somatic dominant to tan (the wild type).

Discussion.

The result of the experiments previously described shows that these somatic modifications in *Bruchus*, which concern the elytra of the females, are not due to factor mutations in the germ cells, because they are not transmitted. It seems, therefore, that the best interpretation is to assume that they are somatic mutations. Ordinary somatic variations are modifications that are supposed to be caused by environmental factors, as they are not inherited. They always display a normal variability curve. The converse of this is true, for these mosaics, since they manifest a sharp discontinuous variation, not unlike a mutation. Mutations have usually been described as discontinuous variations that breed true. A mutation that occurred in a germ cell in an animal would breed true because

of the necessity of sexual reproduction, but a mutation that took place in a somatic cell could be discontinuous, although it would not be transmitted sexually. This reasoning led the author to suggest that these elytral mosaics in *Bruchus* should be called somatic mutations.

In plants mutations may occur in the germ cells or in any meristematic tissue. According to Babcock and Clausen ('18), "Factor mutations in meristematic cells, or vegetative mutations, as distinguished from those originating in the germ cells, give rise to simple *bud sports* or to *chimeras* according to the location of the mutating cells. A bud sport is a shoot or branch which differs genotypically in one or more characters from the remainder of the plant. Here the factor mutation must occur in one of the undifferentiated cells of the very young shoot. Just as in the case of factor mutations in germ cells, so in vegetative mutations the somatic effects range from single visible character differences to manifold effects in which many structural details are different." The fact that somatic factor mutations do occur in plants seems to be well established, and, furthermore, these somatic factor mutations display the same order of dominance, as manifested by factor mutations, which occur in germ cells. Although somatic factor mutations are extremely rare in animals as compared to this same behavior in plants (because the latter can be propagated, while the former can not be), this, in itself, is not sufficient argument to discredit the idea that the same phenomena are involved. It appears, therefore, in this relation that the mosaics in *Bruchus* are not unlike somatic mutations in plants. Emerson ('21) has lately noted similar phenomena in maize.

The ordinary gynandromorph, as described by Morgan and Bridges ('19), is an animal that shows some male characters on one side of the body and female on the other, but the elytral mosaics in *Bruchus* are not gynandromorphs in this sense because the sexual organs are not modified, every mosaic being a normal female. These authors ('19) further showed that sex-linked characters are usually involved in the gynandromorphs of *Drosophila*. There is evidence that nearly all gynandromorphs are potentially females, but that a sex-mosaic results through the elimination of one sex-chromosome immediately after fertilization; this is due to

the fact that the normal female in *Drosophila* has two (XX) sex-chromosomes in her body, but if one is eliminated on one side of her body, male characters will then develop on that side. There is in *Drosophila* no essential difference between gynandromorphs (sex-mosaics) and somatic mosaics, except that the former concern the elimination of one sex-chromosome, while the latter involve the elimination of an autosome. The mechanism of elimination, too, is the same.

It is true of *Bruchus* that all visible mosaics were females, but the author ('21) has shown that these color factors in this insect are not sex-linked, but are sex-limited, autosomal traits. It is improbable that any change in the X-chromosome could produce these mosaics. Morgan and Bridges ('19) have shown that gynandromorphs are neither caused by partial fertilization, as first suggested by Boveri, nor due to polyspermy, as first interpreted by Morgan, but are caused by chromosome elimination. Chromosome elimination, as described by the above writers, means that during some early stage of development of the embryo one of the daughter sex-chromosomes fails to pass over to the daughter plate, and thereby becomes eliminated from the nucleus. Relative to the mosaics of *Bruchus,* it is impossible to account for their appearance through the elimination of the X-chromosome, because these characters involve the autosome in which the allelomorph series for the R (red) gene is located, and not the X-chromosome.

Morgan and Bridges ('19) account for mosaics of *Drosophila* by chromosomal elimination; in one case they tried to account for the origin of a mosaic, as produced from binucleated eggs, because each paternal nucleus was known to have had a different ancestry. It is unnecessary to make this latter assumption for these mosaics of *Bruchus,* because both nuclei of a binucleated egg (if such could occur) would of necessity be alike, and when fertilized by a normal sperm, all of the offspring would be homozygous, unless it is assumed that a mutation occurred in one nucleus before fertilization, and in that event the effect would not be localized in a single wing, but would involve half the entire body.

In *Drosophila* nearly all mosaics can be interpreted as caused by chromosome elimination, because the homologous chromosome from one parent carries factors that are different from its mate.

If these mosaics of *Bruchus* had originated from heterozygous cultures, then the elimination of one autosome might produce one elytrum of one color and the other of a different one; but since these mosaics came from homozygous cultures, it is evident that the elimination of an autosome previous to or even after fertilization could not have this effect. It is difficult to conceive of any kind of autosomal non-disjunction that could possibly create a red elytrum, for example, when the insect was pure for black. Neither can non-disjunction nor the elimination of an autosome have any effect in causing this kind of a mosaic unless one assumes that a mutation occurs after fertilization. The evidence is in favor of this interpretation because these mosaics are not transmitted. It appears, therefore, more reasonable to assume that a mutation has occurred in one autosome during the embryonic development of the insect. Neither is it necessary to assume that autosome elimination or non-disjunction is essential to account for these mosaics even after a somatic mutation has taken place, unless these mosaics were recessives instead of dominants.

Normally recessive mosaics could not be seen, even though they occurred frequently, because a recessive autosome mutation could not be visible in the F_1 progeny, except through autosome elimination. To illustrate the appearance of such a mosaic, let us suppose in a pure culture for red that a female was found with one black elytrum (black is recessive to red). The normal autosome complex on each side of the body of a homozygous red female would be R R. If a mutation to black would occur in one autosome on her right side (R^b is the formula for black), then her right side would be R R^b, and her left side, normal, R R; but because red is dominant to black, when heterozygous, she would still appear red unless the autosome carrying R (red) on her right side would be eliminated, then one side would be black and the other red.

The time in the ontogeny at which the mutation occurred would govern the extent of its effects. If it took place at the first cleavages, for instance, the entire half of the body might be affected; but if it took place later at the time when the *anlagen* of the wings are differentiated, then one elytrum would be black and the other red.

It is true, however, for every mosaic so far discovered in

Bruchus that only elytral colors which are dominant to it have been found, so it is essential to apply next the elimination mechanism to the actual mosaics observed in this insect.

The most common mosaic in this beetle is a red elytrum and a black body originating from pure black stock. This condition is accounted for by assuming a mutation to red in one autosome. Let us now suppose in a normal female, homozygous for black, that, previous to the formation of an elytrum, one autosome mutated to red; the autosomes for the normal elytrum, therefore, would be R^bR^b (pure black), while the mosaic elytrum autosomes R^bR (heterozygous for black and red). Since red is dominant to black, the one R on the one side of this insect is sufficient to make this elytrum red; therefore, a mosaic results as was observed for *Bruchus*. (This is true for the thirty-one mosaics found.) Now, let us apply autosomal elimination for this mosaic; if the autosome R^b (black) was eliminated, the proper mosaic would result; but, on the other hand, if the autosome carrying R (red) was eliminated, the insect would not be a mosaic, but a normal, homozygous, black individual.

Again, if the mutation occurred in two autosomes (a very improbable assumption), it could cause the following mosaic from a homozygous, black insect. Supposing the autosomes on the right side of its body are normal, R^bR^b (black right elytrum), but on the left side a mutation to red occurred in each autosome, then the autosomes for its right elytrum would be R R (pure red). This insect would then have a black right elytrum and a red left one; in this case elimination could not change the mosaic.

The first interpretation with reference to dominant autosome mutations appears correct for these mosaics of *Bruchus*. It seems, therefore, that to account for these mosaics autosomal elimination is not essential, unless a mosaic is visible which is recessive to the normal; in that case autosome elimination would be necessary.

The most plausible explanation, then, is to regard these thirty-one dominant, elytral mosaics of *Bruchus* as somatic mutations that originate in an autosome on one side of the body some time during its ontogeny. The results as described in this paper for *Bruchus* agree with Morgan and Bridges ('19) relative to dominant mutations in somatic tissue. "The general evidence from mutations in

Drosophila," to quote these authors, "makes it highly probable that when a mutation occurs it takes place in only one chromosome of the pair. Hence any mutation in somatic tissue, if recessive, would be concealed by the presence of the normal allelomorph in the homologous chromosome." This is the key to the situation for *Bruchus,* since these thirty-one mosaics appear through dominant somatic mutations in one chromosome of the pair.

It is evident that these somatic mutations in *Bruchus* concern the autosome in which the multiple allelomorph gene for R (red) is located. The difference between the factor mutations which the author ('21) discovered for the body and elytral color factors located at the locus, R, in this autosome and its somatic mutations as manifested in these elytral mosaics is that the former are transmitted, while the latter are not.

The evidence further indicates that there is a chromosome continuity between the gene for R (red) in this autosome in the germ cell and this same gene as manifested through the thirty-one mosaics in *Bruchus.* Of these somatic mutations, twenty-two occurred in homozygous black cultures, through a mutation from recessive black to dominant red; only one from a pure white culture, through a mutation from recessive white to dominant black; while eight appeared from wild stock, seven of these mutated from recessive tan to dominant black, and one of these from recessive tan to dominant white.

The most noteworthy result is that germinal mutations and somatic mutations are identical factor mutations, because both originate through a mutation in a chromosome. This paper furnishes evidence in favor of the chromosome hypothesis.

The conditions noted in these unilateral somatic mosaics perhaps suggest that the chromosome mechanism may also be found to account more generally for the bilateral type of body symmetry, at least in certain groups. Conklin has pointed out that while types of cleavage, symmetry, etc., are determined by cytoplasmic influences, this is not at variance with the chromosome doctrine, since the cytoplasm is itself influenced by the nucleus through the large amount of nuclear material which escapes at every mitosis. The investigations regarding cytoplasmic localization and their part in inheritance merely shows "that in early development inherited

characteristics, like material substances, are chiefly derived from the mother." The facts upon which are based the conclusions recently drawn from a study in asymmetry in *Peromyscus* by Sumner and Huestis ('21) are not necessarily contradictory to Conklin's point of view, in spite of their statement that "the chromosome mechanism of heredity . . . is ill-adapted to account for the transmission of definite spatial relationships. . . ." Future evidence bearing upon the question of symmetry will be very welcome.

Summary and Conclusions.

1. During the years 1918 to 1920, among the thousands of Bruchid insects examined, thirty-one elytral mosaic females were found in homozygous cultures of the black, white, and tan stocks. The usual expectancy from pure cultures is that the elytra of the normal female will be red-red for the red mutant, black-black for the black one, white-white for the white mutant, and tan-tan for the wild type, but such mosaic types as red-black, black-red, black-white, black-tan, tan-black, and white-tan have appeared from homozygous cultures. These display the same order of dominance as was discovered by the author ('21) for the four body colors (red, black, white, and tan or the wild type).

2. Chromosome elimination of any kind is not essential to account for these mosaics, but a dominant somatic mutation in one chromosome of the pair is the most plausible explanation. The time in the ontogeny at which the mutation occurred would govern the extent of its effects; the earlier that it took place, the greater its effect. It is evident that it did occur at the time when the *anlagen* of the wings were differentiated, otherwise an elytral mosaic would not have appeared.

3. The difference between the factor mutations which the author ('21) discovered for the body and elytral color factors located at the locus R (red), in an autosome and its somatic mutations as manifested in these elytral mosaics, is that the former are transmitted, while the latter are not. There appears to be a somatic continuity, however, between this gene for R (red) in this autosome in the germ cell and this same gene as manifested through its thirty-one somatic mutations.

4. Of these thirty-one somatic mutations, twenty-two occurred through a mutation from homozygous, recessive black to dominant red; one through a mutation from homozygous, recessive white to a dominant black; while eight originated in wild cultures, seven of these mutated from recessive tan to dominant black, and one of these from recessive tan to dominant white.

5. In conclusion, the most noteworthy result is that somatic and germinal mutations are identical in that both are due to mutations originating in a chromosome. The time in ontogeny at which the mutation occurs determines whether it appears as germinal or merely as somatic.

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NOTES ON PSITHYRUS, WITH RECORDS OF TWO NEW AMERICAN HOSTS.[1]

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In Franklin's monumental work, "The Bombidæ of the New World," we have a taxonomic outline of the American bumblebees, equal, if not superior, to similar works on the European species. One is rather disappointed, however, on comparing our knowledge, or rather lack of knowledge, concerning the habits of the American species with the interesting accounts of the habits of the European species given by men like Hoffer and Sladen. What we do know about the habits of our North American species we owe chiefly to the efforts of Putnam, Franklin, and Frison, but the field is so large that the surface has hardly been scratched. This is especially true of one of the subdivisions of our Bremidæ,[2] the genus *Psithyrus*. Of the 13 or 14 species of *Psithyrus* described from the New World, the hosts of only two have thus far been recorded. On July 7, 1914, Sladen ('15), at Agassiz, British Columbia, dug up a nest of *Bremus flavifrons* Cresson, victimized by *Psithyrus insularis* Smith, and two years later Frison ('16) recorded that, during the summers of 1910 and 1915, he had repeatedly found the nests of *Bremus pennsylvanicus* De Geer infested by *Psithyrus variabilis* Cresson. To these two records the writer wishes to add two others, those for *Psithyrus laboriosus* Fabricius and *Psithyrus ashtoni* Cresson.

Before going into detail, however, it seems desirable to give a brief résumé of the structure, life history, and habits of these social parasites, as we know them chiefly from the work of Kirby (1802),

[1] Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University, No. 201.

[2] In using *Bremus* and *Bremidæ* instead of the familiar *Bombus* and *Bombidæ*, the writer follows Frison ('19) who in a recent paper pointed out the reasons for making this change (cf. also the extensive paper by Morice and Durrant ('14) on this subject).

Smith ('55), Hoffer ('81, '88), Sladen ('99, '12, '15), and Frison ('16, '21).

Structure.

Both sexes of the genus *Psithyrus* so closely resemble those of the genus *Bremus* that anyone, other than a specialist, would see no difference between them; and even the specialist sometimes has difficulty in determining whether a certain male is a *Bremus* or a *Psithyrus,* so that it has occasionally happened that *Bremus* males have been described as *Psithyri,* and *vice versa.* It is quite easy, however, to tell a *Bremus* female from a *Psithyrus,* because the latter lacks *corbiculæ,* or pollen baskets, a fact which was first noticed by Kirby (1802, I., pp. 209, 210). Illiger (1806, p. 173), suspecting a corresponding difference in habits, separated them from the true bumble-bees, and Newman ('34, p. 404) later gave the group the generic name of *Apathus.* This term held sway for over 40 years, when it was discovered that the name *Psithyrus* had been given to the group by Lepeletier in 1832 (p. 273).

Life History and Habits.

According to Hoffer ('88) and Sladen ('12), the young *Psithyrus* queen, like the young *Bremus* queen, hibernates in the ground, but reappears somewhat later in spring than does the latter. Having no apparatus for collecting pollen, she is unable to found a colony of her own, as does the *Bremus* queen, but, like the European cuckoo and some of our American cowbirds, perpetuates her kind by entering, and laying her eggs in, the nests of her more industrious cousins of the genus *Bremus.* The latter, like the dupes of the European cuckoo, rear the larvæ of these lazy guests instead of their own. As will be seen later, these latter, at least those in the earlier stages of development, are probably systematically destroyed by the *Psithyrus.*

That the *Psithyrus* queen does not always gain admittance to a *Bremus* colony without a struggle is indicated by the frequent discovery in bumble-bee nests of dead or disabled *Psithyri* or *Bremi,* or both, and is confirmed by direct observation when a *Psithyrus* first enters, or is placed in, a *Bremus* nest. In these encounters the *Psithyrus* has a great advantage over the individual members

of the *Bremus* colony. She has a powerful sting, and her integument is so thick that her opponents are unable to penetrate it with their stings. But, like Siegfried and Achilles, the *Psithyrus* is vulnerable in certain places—*e.g.*, the neck—and it is chiefly for this reason that she is not always successful.

However, as already indicated, a *Psithyrus* queen sometimes does gain admittance to a *Bremus* colony without losing her life and may remain with the colony until she dies. Here the question arises: What is the attitude of such a *Psithyrus* queen and the rightful owners of the nest to each other? The two foremost European authorities on the subject do not agree on this point. Hoffer ('81, '88), who studied the habits of the European *Psithyri* more extensively than any other investigator, repeatedly found the *Psithyrus* queen living peacefully with the members of the *Bremus* colony, including the host queen. Sladen ('99, '12, '15), on the other hand, claims that the *Psithyrus* queen, at least those of *Psithyrus rupestris* Fabricius and *Psithyrus vestalis* Foucrier, always kills the *Bremus* queen,[1] either at the time the *Psithyrus* queen enters the nest or a few days later, when the *Psithyrus* is about to begin egg-laying. After the *Psithyrus* has committed this murder, the *Bremus* workers, according to Sladen ('12), constantly watch for an opportunity to avenge their mother by killing the usurper. He (p. 60 ff.) very vividly describes this phase in the life history of the *Psithyrus* queen and the afflicted bumble-bee colony as follows: "It is the practice of the *Psithyrus* female to enter the nest of the *Bombus*, to sting the queen to death, and then to get the poor workers to rear her young instead of their own brothers and sisters.

"The way in which the *Psithyrus* queen proceeds in order to ensure the success of her atrocious work has all the appearance of a cunning plan, cleverly conceived and carried out by one who not only is a mistress of the crime of murder, but also knows how to commit it at the most advantageous time for herself and her future children, compelling the poor orphans she creates to become her

[1] On July 14, 1911, Sladen ('12, p. 70) took a nest of *Bremus hortorum* Linnæus, containing 49 workers of *B. hortorum*, and 16 young queens and 2 males of *Psithyrus barbutellus* Kirby. From this rather meager evidence he concludes that *Ps. barbutellus* is probably "parasitic in the same deadly way as *Ps. rupestris* and *vestalis*."

willing slaves. . . . Her first care is to ingratiate herself with the inhabitants, and in this she succeeds so well that the workers soon cease to show any hostility towards her. Even the queen grows accustomed to the presence of the stranger, and her alarm disappears, but it is succeeded by a kind of despondency. Her interest and pleasure in the brood seem less, and so depressed is she that one can fancy she has a presentiment of the fate that awaits her. It is by no means a cheerful family, and the gloom of impending disaster seems to hang over it."

The hostile attitude of the *Bremus* workers toward the *Psithyrus* which has killed their mother (in one case weeks before) is described by Sladen ('12, pp. 256, 277) as follows: "It was clear that the workers deposed the *Psithyrus* queen, and I think that this was the culminating act in a revolt that the queen had all along found it difficult to repress. . . . It appears that when the [*Bremus*] colony is populous the [*Psithyrus*] *rupestris* [queen] will lose her life unless she maintains constantly her rule of repression."

Though interesting, this account of the behavior of the *Psithyrus* queen and the victimized *Bremus* colony toward each other is not only opposed by the earlier work of Smith ('55, p. 210) and Hoffer ('81, '88), but also by recent observations on the habits of some of our American *Psithyri*.

During the summer (June 24 to August 12) of 1921 the writer located 14 *Bremus* colonies, 13 of which were placed in observation boxes a day or two after they were discovered. They were then transferred to the Bussey Institution, where they were kept under observation for periods varying from one to four months. Each box was provided with a glass cover and an opening which communicated with the outside world through holes in boards placed below the screens of three of the windows (one on the second and two on the third floor) of one of the Bussey buildings. Nearly all of the colonies flourished, some producing hundreds of young queens and males. On August 9, colony No. 13 (*B. affinis*) was dug up after much effort, the tunnel being over seven feet in length. The nest was about three feet below the surface and contained the old queen and about 100 workers of *Bremus affinis* Cresson, and the old queen, 3 young queens, and 6 males of

Psithyrus ashtoni. The comb consisted of numerous empty *Bremus* and *Psithyrus* cocoons and a large quantity of *Psithyrus* brood in various stages of development. This colony was kept under observation until September 26. From August 9 until September 14 the nest was examined at least twice a day. Once a week every worker was caught, so that the young *Psithyri,* which had hatched during the preceding week, could be collected without incurring the wrath of the colony. Thereupon the young *Psithyri* were placed in a box and fed with honey and pollen until their pile attained its full color. In this way 29 males and 61 females of *Ps. ashtoni* were collected from this *Bremus-Psithyrus* colony, but a considerable number of males, and perhaps a few females, probably made their escape during the last few days preceding the weekly collections.

Despite the fact that the abdomen of the *Bremus* queen was much distended, and that she was seen to lay eggs on August 10 and on subsequent days, not a single *Bremus* queen, male, or worker hatched. As the nights became colder during the early part of September, the *affinis-ashtoni* colony, which by this time had dwindled down to about a dozen individuals, gradually died off. On September 12 the old *Psithyrus* queen was found dead in the nest, two days later the *Bremus* queen was missing, and the last worker died on September 26.

In this case the *Psithyrus* queen must have gained admittance to the *affinis* nest during or before the first few days of July. The *Psithyrus* and the *Bremus* queen, therefore, lived together for at least two months. During the time they were under observation (August 9 to September 12) the two queens did not show the least antagonism, nor did any of the other members of this *Bremus-Psithyrus* colony exhibit the slightest sign of hostility toward each other.

The fact that no young bees developed from the eggs laid by the *Bremus* queen corroborates similar observations by Hoffer ('88)[1]

[1] This author ('88, p. 101) reports two exceptions to this rule. On September 1, 1880, he took home a nest of *Bremus variabilis* Schmiedeknecht containing the old queen and 15 workers of *B. variabilis,* and 8 females and 10 males of *Psithyrus campestris* Panzer. During the next few days, 4 more males and 9 females of *Ps. campestris,* and 2 males and 3 females of *B.*

and Sladen ('12). Here the question arises: What becomes of the eggs laid by the *Bremus* queen, or workers, of a *Psithyrus*-ridden nest? Hoffer ('88) considers it probable that the young larvæ which hatch from them are eaten by the young *Psithyrus* larvæ. However, Sladen ('12) actually saw a *Psithyrus* queen devour the eggs of *Bremus* workers, and believes that she always disposes of them in this way. He (p. 257) also thinks it is probable that the race-suicidal habit of the workers of *Bremus lapidarius* Linnæus, which sometimes eat the newly laid eggs of their mother, is associated with the parasitism of *Psithyrus*. He believes that the workers which devour the eggs of their stepmother perpetuate this instinct through their sons. In support of this theory, he states that he has never seen the workers of *Bremus latreillellus* Kirby, "a species that is not preyed upon by any species of *Psithyrus*," molest their mother's eggs. This explanation does not seem plausible. It is a well-known fact (cf. Wheeler, '10) that both queen and worker ants, even of those species which are not molested by parasitic ants, sometimes eat their own eggs. Moreover, the writer has frequently seen the workers of *Bremus fervidus* Fabricius eat their mother's eggs, and this species, as will be shown in another paper, probably does not suffer any species of *Psithyrus* to breed in its nests, a view which is supported by more than twenty records (10 by Putnam, "a large number" by Franklin, and 7 by the writer) of *fervidus* nests, none of which were victimized by a *Psithyrus*.

The question has sometimes been raised as to whether or not the members of the genus *Psithyrus* have any workers. Hoffer ('88, p. 114) answers this question negatively. Among the 61 *ashtoni* females reared by the writer there is a great variation in size (cf. Plate I.), some specimens not exceeding that of medium-sized *affinis* workers. It will also be noticed (cf. Plate I.) that the *Psithyrus* males vary greatly in size. If it is true that the difference between queen and worker of the social *Hymenoptera* is due to a quantitative or qualitative difference in feeding during the

variabilis hatched from this colony. Four years later (June 24, 1884), one of his sons discovered a nest of *Bremus pratorum* Linnæus, containing the old queen, 2 young queens, 5 males, and 26 workers of *B. pratorum*, and the old queen, 9 young queens, and 4 males of *Psithyrus quadricolor* Lepeletier.

larval stage, then these small *Psithyrus* females are comparable to *Bremus* workers. Whether or not they are more inclined to work than the full-sized *Psithyrus* females remains to be determined.

Sladen ('12, pp. 62, 63) believes that the *Psithyrus* queen, like a dog, is largely guided by scent in locating the nests of her victims, and is much more likely to find them when they have a short tunnel. In support of this view, he points out that most of the nests containing *Psithyri* which he dug up had tunnels not exceeding fifteen inches in length, and that in no case were they more than two feet long, and he thinks it is probable that species like *Bremus terrestris* Linnæus and *Bremus lapidarius* often have longer tunnels than other species in order to escape the *Psithyrus*. That this precaution is no absolute insurance against the invasion of *Psithyrus ashtoni* is indicated by the *affinis-ashtoni* nest with a tunnel of more than seven feet.

In a recent paper Frison ('19), on the basis of geographical distribution, expressed the belief that *Ps. laboriosus* "is an inquiline in the nests of *B. fervidus* or *B. vagans*." The writer is pleased to be able to confirm the last part of this prediction. On August 2, colony No. 11 (*B. vagans*) was dug up and transferred to a third-story window in one of the Bussey buildings. Eight days later (August 10) the writer found that a *Ps. laboriosus* queen had gained admittance to the nest. At this time the *vagans* colony consisted of the old queen, about 50 workers, and several males. In the afternoon of the same day the *Psithyrus* queen was observed in the act of tearing open a mass of wax containing small *Bremus* larvæ, so that three of the latter rolled to the bottom of the nest box, leaving the remaining four widely exposed. As usual, these larvæ were thrown out of the nest a few minutes later by the *Bremus* workers. About 5 P.M. the *Psithyrus* queen began to gather wax from the cocoons and built a small cell in which, about 7 P.M., she laid several eggs. As each egg was deposited the sting of the *Psithyrus* penetrated the wall of the cell.

Hoffer ('88), who, despite his extensive observations, never saw a *Psithyrus* oviposit, states (p. 100) that the *Psithyrus* queen lays her eggs in pollen masses in which *Bremus* eggs, or larvæ, are already present. This, as is shown by the observations of Sladen ('12) and of the writer, is not true of *Ps. rupestris* and *Ps. labori-*

osus, and, judging from the oviposition of the members of the genus *Bremus,* is rather improbable of any of the *Psithyri.* The observations of Sladen ('12) and of the writer also indicate that Hoffer's ('88) surmise that the *Psithyrus* larvæ, at least those recently hatched, devour the larvæ of their host,[1] is not true of all, if of any, members of the genus *Psithyrus.*

Six of the eggs laid by the *Ps. laboriosus* queen developed into queen larvæ which spun their cocoons about August 25. On September 7, a *Ps. laboriosus* queen emerged from one of these. The queens in the other five cocoons were heavily parasitized by *Melittobia* sp. and did not hatch. Nor did any of the *Bremus* eggs and larvæ develop which were present in the nest when the *Psithyrus* queen gained admittance to this colony. Whether or not, in this case, the *Bremus* queen laid any eggs[2] after the appearance of the *Psithyrus,* could not be determined.

Hoffer ('88, pp. 104, 105) states that whenever the old *Psithyrus* queen dies, while her larvæ are still very young, the latter, as a rule, likewise perish, and he considers it probable that the *Psithyrus* queen forages and contributes something to the support of her offspring, at least during their early larval stages. The latter is not true of *Psithyrus ashtoni,* and, as the observations of Sladen ('12, p. 65) and of the writer indicate, is also improbable of other species. Both the *Ps. laboriosus* and the *Ps. ashtoni* queen were always at home, and, as regards the latter, foraging was out of the question. She was able to fly but a few feet at the time the *affinis-ashtoni* colony was taken, and still her brood, including some newly hatched larvæ, was in a flourishing condition. It is probable, therefore, that the *Psithyrus* larvæ which Hoffer ('88) had under observation died from other causes.

The behavior of the *Psithyrus laboriosus* queen and the *vagans* colony to each other was the same as that of the members of the *affinis-ashtoni* colony, except during the first three or four days.

[1] This is true of the larvæ of certain solitary parasitic bees, as Verhoeff ('92), Höppner ('04), and Graenicher ('05) have shown.

[2] Sladen ('12, p. 69) claims that a large number of workers in a *Psithyrus*-ridden colony become fertile. This was true of my queenless *B. impatiens* colonies, but none of these would accept a *Psithyrus* queen. However, in my *Psithyrus*-infested colonies, all of which possessed the old *Bremus* queen, none of the workers took to egg-laying.

On the first day (August 10) the *laboriosus* queen seized nearly every worker with which she came in contact and rolled the latter toward the ventral side of her abdomen, and made movements as if to sting her victim. This mauling, as a rule, lasted for only a few seconds, when the worker, which in every case was absolutely passive, was again released. In seizing the workers, both the mandibles and the first pair of legs were usually employed simultaneously, but on one occasion a worker was first lifted up by the pile of its thorax with the mandibles and then rolled below the body of the *Psithyrus*. None of the workers seemed to be any the worse for this mauling.[1]

On the second day (August 11) the *Psithyrus* queen only occasionally seized a worker and treated it in the manner described. On the third day (August 12) this rough treatment of the *Bremus* workers became still less frequent, and thereafter the behavior of the *Psithyrus* queen and the *vagans* workers was quite peaceful.[2]

The attitude of the *Psithyrus* and the *Bremus* queen toward each other was somewhat different. From the very start the *Psithyrus* paid little or no attention to the latter, but during the first few days the *Bremus* queen avoided her rival whenever they met, and usually turned the tip of her abdomen toward the *Psithyrus*, as if to ward off an attack. However, these signs of hostility on the part of the *Bremus* queen gradually decreased and ceased completely after the fourth or fifth day, and thereafter both queens lived quite peacefully together.

To the *vagans* males, of which several were present when the *Psithyrus* first entered the nest, and to two young *vagans* queens which hatched on September 15, the *Psithyrus* paid no attention whatsoever, nor did any of these young queens and males exhibit the slightest sign of fear toward the *Psithyrus*.

[1] Sladen ('12, p. 253) who observed a *Ps. rupestris* queen, which he had placed in a *B. lapidarius* nest, treat the *lapidarius* workers in a manner similar to that described above, concluded that the workers were too small to get hurt, but, as will be seen later, this is not an adequate explanation.

[2] This agrees with similar observations by Smith ('55, p. 210) and Wheeler ('04, p. 353). According to the former, the *Psithyri* "live on the most friendly terms with the industrious part of the community," and the latter, in the case of ants, found that "the relations between the [*Formica*] *consocians* queen and the *incerta* workers were perfectly cordial."

As in the case of the *affinis-ashtoni* colony, the members of the *vagans-laboriosus* colony became more and more inactive as the weather grew colder. On September 12 the old *vagans* queen disappeared from the nest, and four days later the old *Psithyrus* vanished. The two had lived together for about five weeks.

From what has been said it is evident that *Ps. ashtoni* and *Ps. laboriosus* do not always, if ever, kill the host queen, and in this respect they behave like the two other American *Psithyri* whose hosts are known. One of these, *Ps. insularis,* was found breeding in a colony of *B. flavifrons* by Sladen ('15), and the other, *Ps. variabilis,* was found repeatedly in the nests of *B. pennsylvanicus* by Frison ('16). In the six infested nests reported by these two authors, each one contained the old *Bremus* queen, and this despite the fact that in both cases young *Psithyri* had emerged, or were about to emerge.

All of these observations on the habits of our American *Psithyri* agree with Hoffer's ('81, '88) account[1] and make Sladen's claim (that *Psithyrus rupestris* and *vestalis* always kill the host queen) extremely doubtful. Sladen ('12) based his conclusions on the fact that he never found a living *Bremus* queen in a *Psithyrus*-ridden nest.[2] Against this we have the positive evidence of Hoffer ('88, pp. 126, 148), who, as in the case of *Ps. campestris, quadricolor,* and *barbutellus,* also found the queens of *Ps. rupestris* and *vestalis* living in peace with the host queen, in one case after some of the offspring of the *Psithyrus* had emerged. Further inquiry into the queen-killing habit of *Ps. rupestris* and *vestalis* will undoubtedly show that Sladen ('99, '12, '15) based his conclusions on insufficient evidence.

This same criticism applies to another of Sladen's ('12) conclusions. He (p. 68) believes that *Psithyrus* queens do not kill

[1] Recent observations by Wheeler and Taylor ('21) indicate that *Vespa arctica* Rohwer, which has *Psithyrus*-like habits, being a social parasite on *Vespa diabolica* De Saussure, may also sometimes, if not always, live in peace with the host queen.

[2] Such negative evidence, as the work of Wheeler and Taylor ('21) indicates, is very unsatisfactory. Of nine *Vespa diabolica* nests taken by these two authors comparatively early in the season (before August 4), only one contained the old *diabolica* queen, and yet, with a single exception, these nests were not parasitized by *Vespa arctica.*

one another, because he never found a dead *Psithyrus* in a nest ruled by a *Psithyrus*. This certainly is not true of *Ps. laboriosus*. During June, July, and early August, *Ps. laboriosus* queens were very common in the vicinity of Boston, so that numerous experiments along this line could be carried out. The writer repeatedly caught *laboriosus* queens which were searching for *Bremus* nests and placed them in a small box containing comb filled with honey. Whenever two such queens were placed in this box, or in similar receptacles without comb, they immediately engaged in a violent battle which invariably resulted in the death of one of the combatants.

Psithyrus laboriosus queens also repeatedly appeared in several colonies of *Bremus fervidus* Fabricius and two of *Bremus bimaculatus* Cresson, one consisting of the old queen and about 25 workers, and the other of the old queen, several young queens, and about 50 workers. All of these colonies were kept on the third floor of one of the Bussey buildings. The *fervidus* colonies always expelled these intruders by a unique and very effective method, which will be described in another paper. The two *bimaculatus* colonies, on the other hand, never seriously objected to these intruders, and the latter sometimes stayed in their nests for several days. However, neither of the *bimaculatus* colonies produced any young *Psithyri*.

Psithyrus laboriosus queens which were searching for bumblebee nests were also frequently placed in these *bimaculatus* colonies. If a *laboriosus* queen was already present in the nest, and another one was introduced, the two *Psithyrus* queens usually clenched immediately, and within a minute or two, sometimes within a few seconds, one toppled over, mortally stung. In one or two cases the introduced *Psithyrus* queen tried to avoid a conflict by making a dash for the flight-hole as soon as she noticed the other *Psithyrus*. The two opponents, as a rule, seized each other by one of the legs and endeavored to sting one another. As soon as one had succeeded in penetrating the body of her adversary with her sting, she attempted to extricate herself from the embrace of her vanquished foe. During these encounters it sometimes happened that legs were torn off, or that the dead *Psithyrus* held firmly to one of the legs of the victor with her mandibles so that the latter had to be

released. Such a *Psithyrus* in turn was sometimes killed a few minutes later by a third *Psithyrus* which was placed in the nest.

The behavior of the *Psithyrus laboriosus* queens toward the *bimaculatus* workers differed essentially from that described for the *Ps. laboriosus* queen in the *vagans* colony. Every one of the 16 *laboriosus* queens used in these experiments completely ignored the *bimaculatus* queens and workers. Only once (shortly after she had been introduced into the nest) was one of these *laboriosus* queens observed raising one of her middle legs threateningly toward a *bimaculatus* worker, a form of intimidation which is quite frequent, even between members of the same *Bremus* colony.

Psithyrus laboriosus and *Psithyrus ashtoni* queens were also frequently placed in two strong colonies of *Bremus impatiens* Cresson, one having about 125 workers and the other more than 450. As soon as a *Psithyrus* queen was introduced into one of these nests, a great uproar arose in the colony. The workers rushed madly in every direction hunting for the source of the disturbance. The *Psithyrus* queen was seized almost immediately by numerous workers who tried to sting her, and was thus made absolutely helpless. A few bellicose workers, unable to get hold of the *Psithyrus,* seized some of the attached workers instead, and then attempted to sting toward the center of the struggling mass. During one of these experiments (August 15) such a fighting mass was lifted out of the nest box with a pair of forceps. When the workers finally released their hold, it was found that the mass had consisted of 17 workers and the *Psithyrus.* The latter and four of the workers were mortally stung. Although the *Psithyrus* made attempts at stinging during this struggle, one of the four workers was stung to death by one of its fellows at the periphery of the mass, and it is probable that the other three met death in the same manner. At the beginning of two of these experiments two workers, in their excitement, attacked each other (several inches from the *Psithyrus*) and one was stung to death. All of these experiments with the *B. impatiens* colonies always ended with the death of the *Psithyrus.*[1]

[1] Frison ('21) reports that he found two *Ps. laboriosus* queens, one dead and one paralyzed, in the nests of *Bremus auricomas* Robertson and *B. pennsylvanicus,* and expresses the belief that both were stung to death by the

However, as will be seen from the following incident, a battle between a *Ps. laboriosus* queen and *B. impatiens* workers may have quite a different ending under somewhat different conditions. On July 24, 19 workers of colony No. 8 (*B. impatiens*), which had been transferred to one of the Bussey buildings on the preceding day, were caught at the old nest site and placed in a small glass jar. A few minutes later a *Ps. laboriosus* queen was discovered on some comb which had been left in the empty nest cavity of colony No. 9 (*B. fervidus*). Just to see what would happen, the *Psithyrus* was also placed in the jar. All of the inmates, including the *Psithyrus,* were ill at ease and tried to escape, but one or two of the *impatiens* workers nevertheless attacked the *Psithyrus* queen as soon as they came in contact with her. The latter now went on the warpath herself. She quickly seized one *impatiens* worker after another, whether attacked by them or not, rolled them below her abdomen and stung them to death. This done, she seemed to feel quite at home in the jar and began to lap up the honey which was oozing out from the bodies of her victims. From what has been said before, it is evident that this encounter would have ended quite differently if it had taken place in the nest of the *impatiens* workers. This, as well as some of his own observations (*e.g.,* '12, p. 277), disproves Sladen's ('12, p. 253) claim, already referred to, that the *B. lapidarius* workers, which were mauled by a *Ps. rupestris,* did not get hurt because they were too small.

During the summer of 1905 Wagner ('07, pp. 77, 78) discovered several nests of *Bremus muscorum* Linnæus at some distance above ground, and concluded that the nest-building instinct of this species, which normally builds on the ground, is in a process of transformation. The cause for this change he ascribes to natural selection brought about by the fact that the colonies of this species are destroyed, in large number, by various species of *Psithyrus.* It is improbable, however, that we are here dealing with a change in instinct. Hoffer ('88, p. 95) records that in the spring of 1886

Bremus queens, both of which he found uninjured. However, judging from the non-aggressive attitude of the queens, and the belligerent behavior of the workers of *Bremus impatiens,* the writer would suggest that the two *Psithyrus* queens were stung to death by the *Bremus* workers, which, according to Frison, were present in both nests.

three queens of *B. lapidarius,* which usually builds underground, started their nests in different parts of his house, and that a few weeks later numerous *Ps. rupestris* queens were hunting for these nests. These observations of Hoffer, and the fact that *Ps. laboriosus* queens repeatedly appeared in the writer's *Bremus* colonies kept in a third-story window (about 30 feet above ground), make it evident that *Psithyrus* queens are able to find bumble-bee nests, even if the latter are located at a considerable altitude. Natural selection in this direction is therefore of little benefit to a *Bremus* species as long as the members of the genus *Psithyrus* retain their acute sense of smell.

How exceedingly keen the olfactory sense of these social parasites actually is[1] can be inferred from the following observation: On July 3, an exceptionally pleasant day, colony No. 2 (*B. bimaculatus*) was dug up. The comb had hardly been exposed when a *Ps. laboriosus* queen swooped down upon it. She was captured and a half a minute later another *laboriosus* queen was buzzing about the comb. This one was also caught; but within another minute a third *laboriosus* queen alighted on the comb, and this despite the fact that not a single *Psithyrus* had been noticed in the vicinity previously.

This sudden appearance of the three *Psithyrus* queens also suggests an error in one of Sladen's ('12) experiments. As already stated, this author believes that the *Bremus* workers of a *Psithyrus*-ridden colony are constantly watching for an opportunity to kill the *Psithyrus,* even if the latter has been living with the colony for a considerable period. The observations on which Sladen ('12) chiefly based his conclusion were briefly as follows: On July 9, 1911, this author (cf. p. 251 ff.) dug up a nest of *B. lapidarius* containing 71 workers and a large amount of *Ps. rupestris* brood, but the *rupestris* queen was nowhere to be seen. Suspecting she had hidden herself in a side hole, he left a lump of cocoons in the nest cavity to attract her, and returning a quarter of an hour later actually found a *rupestris* queen on the comb, and concluded that he was dealing with the same *Psithyrus* queen which had been living in the nest. However, judging from Sladen's ('12, p. 253)

[1] Observations of Latter ('06) and the Raus ('18) indicate that this is also true of some solitary parasitic bees and wasps.

description of the hostile attitude of this *rupestris* queen toward the *lapidarius* workers, it seems probable that he was not dealing with the mother of the *Psithyrus* brood.

According to Hoffer ('81, '88), Friese ('88), and Sladen ('12), each European species of *Psithyrus* breeds only in the nests of certain *Bremus* species. Some—*e.g., Ps. rupestris* and *vestalis*—seem to be restricted to a single host, while others, like *Ps. campestris* and *barbutellus,* have two, or even three, hosts. Whether or not our American *Psithyri* have more than one host still remains to be decided. In determining this question we must understand clearly, however, what we mean by the term "host." While it is true that some of the European *Psithyri—e.g., Ps. rupestris*—breed only in the nests of a certain *Bremus* species, they may nevertheless be found lodging temporarily in the nests of other species. It will therefore be necessary, if we wish to avoid confusion, to restrict the term "host" to those *Bremus* species in whose nests a given species of *Psithyrus* is known to breed successfully. Using this definition of a *Psithyrus* host as a criterion, we thus far know of only one host for each of the four American species of *Psithyrus* whose hosts have been discovered. They are as follows:

TABLE I.

Psithyrus.	Host.
Psithyrus ashtoni Cresson	*Bremus affinis* Cresson
Psithyrus insularis Smith	*Bremus flavifrons* Cresson
Psithyrus laboriosus Fabricius	*Bremus vagans* Smith
Psithyrus variabilis Cresson	*Bremus pennsylvanicus* De Geer

But, as already pointed out by Sladen ('15), the geographical distribution of *Ps. insularis* and its host, *B. flavifrons,* are not identical, and we must therefore assume that *Ps. insularis* has more than one host, or that *B. flavifrons* also occurs in Saskatchewan, Manitoba, Ontario, Quebec, North Dakota, Minnesota, Wisconsin, Michigan, and New York. This second alternative is rather improbable.

Similar, though less extensive, discrepancies in the geographical distribution also exist in the cases of *Ps. ashtoni* and its host, *B. affinis,* and of *Ps. laboriosus* and its host, *B. vagans. Ps. ashtoni* either has more than one host, or *B. affinis* ought to be present on

Prince Edward Island, in New Brunswick, Nova Scotia, Quebec, Manitoba, and Saskatchewan. In this case it is difficult to make any prediction. *B. affinis* occurs in New England, Ontario, and Minnesota, and hence may yet be found in the adjoining eastern and central portions of Canada. This seems all the more probable when we consider that comparatively little collecting has been done in these parts of Canada, and that the number of *Bremus* species known from Illinois has been raised from 10 to 15 during the last few years by the intensive collecting of Frison ('19).

In the case of *Ps. laboriosus* and its host, *B. vagans,* we have to assume either that *Ps. laboriosus* has more than one host or that *B. vagans* is also present in South Carolina and Georgia and on Prince Edward Island. *B. vagans* occurs in Nova Scotia, North Carolina, and Tennessee, and it is therefore probable that it will be reported from the adjoining territories mentioned above.

In the case of *Ps. variabilis* and its host, *B. pennsylvanicus,* the geographical distribution of the former only covers a small portion of that of the latter, and it would therefore not be surprising if *Ps. variabilis* is taken in states from which it has not been reported. In fact, its known geographical distribution makes it almost certain that it occurs in Georgia, Indiana, and South Dakota.

There still remain several other interesting questions in regard to the members of the genus *Psithyrus,* such as origin, similarity in coloration with their hosts, and the frequency with which they breed in *Bremus* nests. The most extensive records which we have as regards the last of these three questions are those for one of the European species, *Ps. campestris.* Hoffer ('88, p. 132) records that he found more than 70 nests of *B. variabilis* and *B. agrorum* victimized by this species. Out of 48 nests of *B. variabilis,* 13 contained *Ps. campestris,* and during several summers in the early 80's about half of the nests of these two *Bremus* species harbored this parasite. In 1910 Frison ('16) opened seven surface nests of *B. pennsylvanicus,* four of which were infested by *Ps. variabilis.* Of the four *B. vagans* nests of which we have records (one each taken by Putnam, Beutenmueller, Franklin, and the writer), only one was later victimized by *Ps. laboriosus,* and of two *B. affinis* nests examined (one by Franklin and one by the writer), one was infested by *Ps. ashtoni,* while *Ps. insularis* was

found breeding in the only nest of *B. flavifrons* on record. These figures indicate that in some places, at least in certain years, about half of the colonies of those *Bremus* species which serve as hosts to certain species of *Psithyrus* are parasitized by the latter.

Some of the European *Psithyri—e.g., Ps. quadricolor* and *rupestris*—closely resemble their respective hosts in coloration, while others, like *Ps. campestris,* have no resemblance to their host whatsoever. In the case of the four American species of *Psithyrus* whose hosts are known we have a similar state of affairs. Between *Ps. laboriosus* and its host, *B. vagans,* there is considerable similarity, especially between *Psithyrus* male and host. On the other hand, there is little or no similarity between *Ps. ashtoni* and *insularis* and their respective hosts, *B. affinis* and *flavifrons,* and between *Ps. variabilis* and its host, *B. pennsylvanicus,* there is practically none.

Hoffer ('88, pp. 115, 116) states that those of the European *Psithyri* which are parasitic on *Bremus* species, which are equally large (queen against queen) or larger than they themselves, resemble their hosts more closely than those *Psithyrus* species which prey on smaller *Bremus* species. This correlation in color and size between parasite and host may be true of the European *Psithyri,* but it does not hold for our American species. According to Franklin ('12), the queens of *Ps. ashtoni* and *variabilis* are both considerably smaller than their hosts, *B. affinis* and *pennsylvanicus,* and still, in this case, there is little or no similarity in coloration between parasite and host. On the other hand, the *Ps. laboriosus* queen is somewhat larger than that of its host, *B. vagans,* and here, especially between *Psithyrus* male and host, we have a considerable degree of similarity.

We now come to the very interesting question as to the origin of the members of the genus *Psithyrus.* Müller ('71), Pérez ('83), Hoffer ('88), Sladen ('12), Lutz ('16), and Wheeler ('19), all of whom have paid more or less attention to this question, are agreed that the members of the genus *Psithyrus* are a degenerate offshoot from the genus *Bremus.*[1] One of these authors (Pérez, '83, pp.

[1] All observations on *Bremus-Psithyrus* colonies indicate that in no case is there a genetic relationship like that suggested by Patterson and Packard-Beresford ('03) for *Vespa austriaca* and *Vespa rufa.* According to this

207–215) believes that the representatives of the genus *Psithyrus,* because of their "perfect homogeneity," could not have come from several *Bremus* species, and, on the basis of structure, tries to show that they have all arisen from a single form which was closely related to *Bremus mastrucatus* Gerstæcker. He believes that in the course of time *Ps. barbutellus, campestris,* and *vestalis* became more differentiated from this original type than the other *Psithyrus* species. Hoffer ('88) and Sladen ('12), on the other hand, believe that there is no evidence for such an assumption, and the last-named author suggests that such resemblances as regards coat-color, etc., between several species of *Psithyrus* and their *Bremus* hosts are "clearly attributable to mimicry or exposure to the same conditions of life and not to ancestry." If we accept this explanation, it is difficult to account for those cases where there is not the slightest similarity between *Psithyrus* and host—*e.g., Ps. campestris* and *Bremus agrorum* Fabricius.

It is no doubt true, as Schmiedeknecht ('07) has pointed out, that in the case of the Bremidæ other criteria, such as length of head, are of greater taxonomic importance than coloration. That the latter is nevertheless of considerable value in determining relationship is indicated by the striking similarity between many of the species of the various English and American *Bremus* and *Psithyrus* groups, as they have been established by Franklin ('12) and Sladen ('12, p. 152), in the one case on the basis of structure, and in the other on the basis of habit (pollen-primers, pollen-storers, and carder-bees).

Using coloration as a basis, the various European and American *Psithyri* can be divided into three groups. The first of these, which may be called the *rupestris* group, is restricted to the Old World. The members of this group strikingly resemble a number of *Bremus* species, likewise restricted to the Old World, with one of which, *B. mastrucatus,* they are very similar in structure, as Pérez ('83) has shown. *Ps. rupestris,* the chief representative of this group, is parasitic on *B. lapidarius,* from which it is difficult to distinguish it.

theory, we should expect the parasitic Hymenoptera to be the ancestors of their hosts, a view which, as Saunders ('03) has pointed out, is difficult to harmonize with certain well-established facts.

Group two, which may be called the *laboriosus* group, and which comprises those *Psithyrus* species which Franklin ('12) has assembled under that name, is restricted to the New World. Here, again, we have a group whose members closely resemble a number of *Bremus* species, also restricted to the New World, one of which, *B. vagans,* serves as host to *Ps. laboriosus,* the chief representative of this group.

The third and largest group, which may be called the *vestalis* group, has representatives in both the Old and the New Worlds. To it belong such European species as *Ps. vestalis, distinctus,* and *quadricolor,* and the members of Franklin's ('12) *ashtoni* and *fernaldæ* groups in America. According to Pérez ('83) and Franklin ('12), many of the representatives of this group are also similar in structure. This group likewise closely resembles a number of *Bremus* species, which in this case are present in both the Old and the New World—*e.g., Bremus occidentalis* Greene in America and *B. latreillellus* and *jonellus* in Europe. The last-named species serves as host of *Ps. quadricolor,* a widely distributed European species belonging to this group.

The great difference in coloration (with a parallel difference in structure) between these three groups, the geographical distribution of those *Bremus* species which resemble them, and the fact that many *Psithyri* are parasitic on similarly colored *Bremus* species, all point to an independent origin of at least three *Psithyrus* groups.[1]

Just how the parasitic habits of the representatives of the genus *Psithyrus* may have originated is suggested by certain observations of Sladen ('99, '12). According to this author, many of the later appearing queens of certain *Bremus* species do not take the trouble to start nests of their own, but enter nests already occupied by their own or other species. In the resulting conflict the foundress of the colony is sometimes killed, and her offspring then assists in rearing the brood of the intruder. He states that this occurs frequently between the queens of *Bremus lucorum* Linnæus and its larger variety, *B. terrestris,* the *lucorum* colony serving as a temporary host. From this temporary social parasitism, as he points

[1] A similar independent origin has been suggested by Wheeler ('10, p. 449) for certain ants which are temporary social parasites on other ants.

out, it is but a step to the permanent social parasitism of the members of the genus *Psithyrus*.[2]

This step in the probable evolution of the permanent social parasitism of *Psithyrus* is found among the ants. As Wheeler ('04, '10, '19) was the first one to point out, there is a large group of ants—*e.g., Formica consocians*—in which the queens are no longer able to found colonies of their own without becoming temporary social parasites on other ants, a form of parasitism which is intermediate between that of *Bremus terrestris* and *Psithyrus*.

Summary and Conclusions.

1. The American *Psithyri,* whose habits have been studied, rarely, if ever, kill the host queen.

2. A *Psithyrus laboriosus* queen which is searching for bumblebee nests never tolerates another such *Ps. laboriosus* queen in the same *Bremus* nest.

3. During the first few days after a *Psithyrus laboriosus* queen has gained admittance to a nest of her host, *Bremus vagans,* the *laboriosus* queen intimidates the workers of the colony by rough treatment. After this period of "getting acquainted," the members of the *vagans-laboriosus* colony show no more hostility toward each other than the members of an uninfested *vagans* colony.

4. The *Psithyrus laboriosus* queen does not always, if ever, lay her eggs in a pollen mass in which *Bremus* eggs, or larvæ, are present, but, like the *Bremus* queen, constructs her own egg-cells and, like the latter, attaches them to one or more cocoons.

5. In *Psithyrus*-infested nests of *Bremus affinis* and *Bremus vagans* the destruction of the eggs, or young larvæ, of the host is probably caused by the *Psithyrus,* and not by her offspring.

6. It is not necessary for the *Psithyrus ashtoni* queen, and probably also not for other *Psithyrus* queens, to forage for her offspring in order that the latter may thrive.

7. The representatives of the genus *Psithyrus* have probably originated from several species of *Bremus* rather than from a single one.

8. Similarity in coloration between *Psithyrus* and *Bremus* spe-

[2] Wheeler ('10, p. 451) considers it probable that the permanently parasitic ants went through a similar evolution.

cies is probably due to genetic affinity, and not to "mimicry or exposure to the same conditions."

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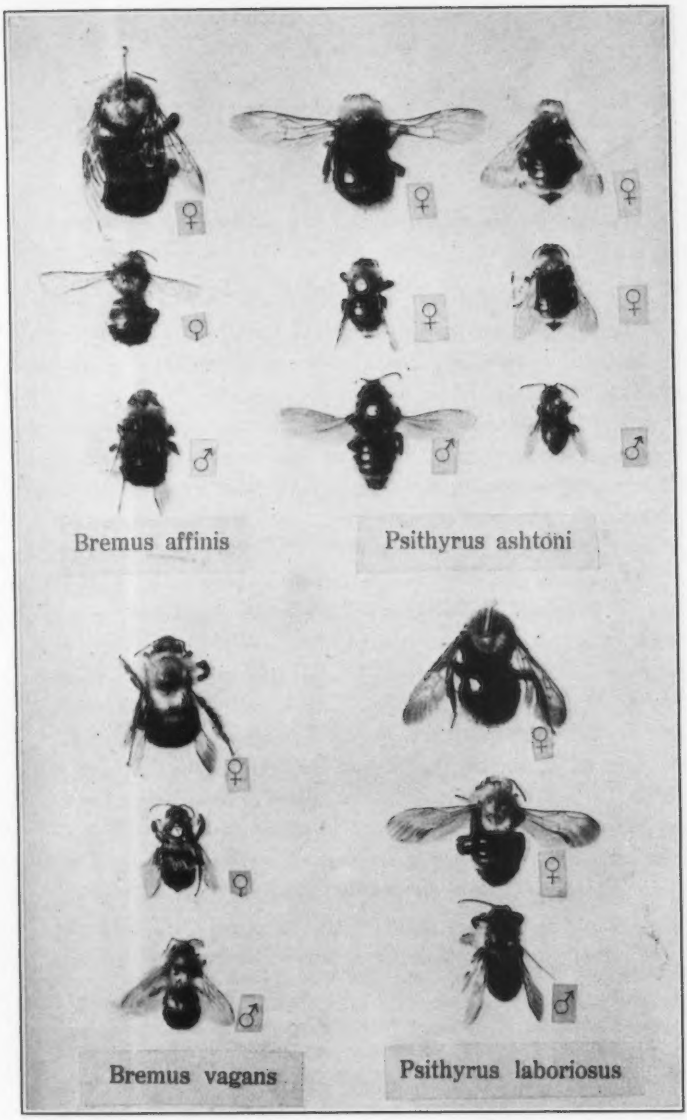
Bremus affinis
Psithyrus ashtoni
Bremus vagans
Psithyrus laboriosus

O. E. PLATH.

THE MULTIPLE TESTIS IN URODELES.

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In a recent paper ("The Interstitial Cells of the Urodele Testis," 1921) the writer referred briefly to the occurrence, in various urodeles, of what might be termed a multiple testis—a testis made up of a series of enlargements, each of which is morphologically and functionally similar to the others or to the simple testis found in the majority of American urodeles. Successive enlargements are separated by constricted regions, often of greater length than the enlargements themselves. Such regions have been referred to by investigators as "sterile." This designation, however, is clearly inapplicable, since, as we shall see, reproductive cells are not absent.

Of the twelve species of American urodeles examined by the writer, multiple testes are found to occur in three—*Desmognathus fusca, Diemyctylus viridescens,* and *Diemyctylus torosus.*[1] Among the urodeles of Europe, according to Champy, axolotl and the tritons exhibit this feature. I myself have examined testes of the European *Salamandra atra* and find them to consist of as many as three enlargements or lobes.[2]

Though the multiple testis is of rather common occurrence, no satisfactory discussion of its origin and significance has yet been encountered in the literature. Following are a few of the most

[1] von Wittich ('53) describes and illustrates (his Fig. 18) the testis of *Necturus lateralis* (*Necturus maculosa*) as consisting of three parts or lobes. Hoffmann ('73–'78) in Bronn's "Klassen und Ordnungen der Amphibien", states that von Wittich also describes the testis of *Menopomă* (*Cryptobranchus allegheniensis*) as of the multiple type. The author has found in *Necturus* no testes such as that illustrated by von Wittich, though about sixty males have been examined; neither has a multiple testis been found in any of the six *Cryptobranchus* males studied.

[2] Each enlargement or secondary testis will in this discussion be termed a lobe, a designation agreeing with that employed by Champy and other workers.

pertinent comments: Kingsbury ('02) noted the occurrence of lobes in the testis of *Desmognathus*. He says: "There seems to be no absolute correlation of this condition with other structural features of the salamander, save that the presence of two or more enlargements occurs more often—in fact, quite constantly—in large animals. A similar division of the testis into 'lobes' occurs in other salamanders with an elongated body, and has been noted in *Amphiuma* and *Spelerpes*.[1] The segmented condition of the organ in *Cœcilians*[2] is perhaps to be associated likewise with the elongated form of the body."

Champy, similarly, comments on the relation of body size to the presence of the multiple type of testis. He believes that the variation in the number of lobes is correlated with that in the size of the species. He found the lobes very numerous in axolotl, more numerous in *Triton cristatus* than in *Triton palmatus* and *Triton punctatus*, and least numerous in the salamanders, though the last-named animals, he concedes, are of rather large size. Champy further considers that among the individuals of any species the number of lobes will be proportional to the size of the animal. He states that animals reared with insufficient food remain small and their testes develop few lobes, while an animal of one year, well nourished, may possess a testis of numerous lobes.

From my own observations on the testes of numerous species of American urodeles it would appear that the general conclusions stated by previous workers are in part inapplicable. The size of the species appears to be in no way correlated with the occurrence of a multiple testis. For example, our largest species, *Cryptobranchus* and *Necturus*, have testes of the simple type; three species of medium size—*Plethodon glutinosus*, *Gyrinophilus porphyriticus*, and *Amblystoma punctatum*—likewise have testes of unit

[1] The writer is unable to confirm the occurrence of a multiple testis in *Spelerpes*, though about a dozen males of the species *Spelerpes bislineatus* have been examined. McGregor ('99) likewise states that no division of the testis into lobes occurs in *Amphiuma*.

[2] The multiple testes of the Cœcilians, judging from the descriptions available, are probably of a type different from those of the *Urodeles*. Spengel ('76) describes the enlargements as being numerous in an immature animal, and states that they are separated by regions from which germ cells are entirely absent. Urodele males show no such primary growth centers; neither are germ cells entirely absent from any region of the testis.

structure. The species possessing the multiple testes (*Desmognathus* and *Diemyctylus*) are, in comparison with the above, small animals, yet their testes may consist of from two to five lobes. Plainly the size of the species is not to be regarded as a factor.

That the elongated form of the body is responsible for the multiple testis is likewise hardly conceivable. Among our American urodeles none are more slender and graceful of body than *Gyrinophilus* and *Spelerpes bislineatus*. Yet in these animals the testis, though an elongated structure, is not divided into lobes. *Desmognathus* and *Diemyctylus*, possessing multiple testes, are comparatively stout-bodied animals.

Champy's conclusion that the number of lobes in males of the same species is proportional to their body length is only in a limited sense correct, as the following figures from my records show: The average length of eight *Desmognathus* males with testes of two lobes each is 9.8 cm.; the average length of six males with testes of three lobes is 10.2 cm. or 0.4 cm. greater. Nevertheless one of the individuals of the first group measured 12.2 cm., while no animal of the second group exceeded a length of 10.5 cm. The average body length for the same group of eight males having testes of two lobes each is 9.8 cm. Within this group, however, are lengths ranging from 8 cm. to 12.2 cm. Clearly other factors than mere body length must be concerned, else we should find much less variation within this group.

Conceding that males which have been kept small by lack of food may develop testes of few lobes, as Champy states, the writer is forced, so far as *Desmognathus* is concerned, to doubt Champy's further claim that animals well fed may become very large, and, at one year of age, possess testes of numerous lobes. Since the urodele male completes but one spermatogenetic cycle annually, the males of one year referred to by Champy would of necessity reach sexual maturity with testes of many lobes already developed. In other words, the multiple testis, whatever its correlations, would develop simply through the initiation of greater growth activity in localized regions of the original cord of germ cells, as suggested by Kingsbury ('02). A male approaching sexual maturity might, on the basis of this theory, possess an even greater number of lobes than one which had been sexually active for several seasons.

Champy, indeed, states his belief that the number of lobes is not at all dependent upon the age of the male.[1] In *Desmognathus*, however, the evidence shows clearly that the sexually immature male, regardless of size and vigor, possesses only a simple testis—*i.e.*, only one lobe is present. The multiple testis, then, must arise by the addition of other lobes through growth processes *following sexual maturity;* in other words, the *age* of the animal must be considered as a factor.

Preceding investigators, so far as can be ascertained, have merely noted the occurrence of the multiple testis and speculated as to its possible correlations. The origin of the numerous enlargements or lobes has not been adequately investigated, and it is to their origin and development that we must turn if we are to fully appreciate their significance. Spengel ('76) makes the brief statement that the numerous lobes of a multiple testis arise as the result of the complex growth, degeneration, and regeneration processes of the organ.[2] This statement, properly elaborated, furnishes, I believe, the correct interpretation of these structures. The pattern of the spermatogenetic processes, in other words, explains the multiple testis. It is clearly not a structure of segmental origin; its correlation with the body size or body form of the species or the individual of the species can not be established. It is, as I shall attempt to show by a discussion of the urodele plan of spermatogenesis, a structure arising from the combined operation of three factors: (1) the slow movement of the spermatogenetic "wave"; (2) the delayed regeneration of the emptied lobules at the close of the spermatogenetic cycle; (3) the age of the animal—*i.e.*, the number of sexual cycles through which the two first-named factors have been operative.

[1] An earlier French worker, Duvernoy ('51), according to Spengel, reached a similar conclusion. Spengel (p. 65) says: "Er glaubt, die Zahl der Abschnitte sei allein abhängig von der Brunst, da er keine constanten Altersunterschiede zu entdecken vermochte."

[2] Spengel (p. 65) says: "Die eingehendere Erörterung dieser Frage muss ich bis zur Darstellung der Entwickelung und des Wachsthums verschieben . . ." Whether Spengel ever published a more detailed explanation of the multiple testis, as the foregoing quotation shows he proposed doing, I am unable to state. No reference to any later work by him on the amphibian testis has been encountered in the literature, although his first publication, "Der anatomische Bau des Urogenitalsystems," is often extensively quoted.

The structure of the urodele testis has been described at length in a previous communication and need be only briefly reviewed at this time. The elongated testis is suspended from the dorsal abdominal wall by a mesorchium in which run the blood vessels and efferent ductules. The latter, varying from few to several in number, lead from the longitudinal collecting duct of the testis to the ductus deferens (Wolffian duct). The longitudinal collecting duct may be superficial in position, or as in many urodeles, more centrally located. The structural units of the testis, the lobules,[1] empty into the longitudinal collecting duct either directly, by means of very short ducts, or more indirectly, by way of longer, much-branched tributary ducts.

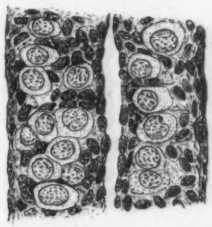

FIG. 1. Longitudinal section of the extreme anterior end (primary germ-cell cord) of a *Desmognathus* testis. The longitudinal collecting duct is shown, bordered by primary spermatogonia. The caudal germ-cell cord remaining after the complete degeneration of the lobules is essentially the same structure, as is also the slender cord intervening between successive lobes. The primary spermatogonia, in these latter structures, are properly termed "residual" spermatogonia.

A study of the lobule itself shows it to be a structure made up of reproductive cells all in approximately the same stage of development. Figure 2 shows the lobules as hollow cyst-like chambers lined by spermatogonia. All the cells in a lobule undergo their developmental transformations synchronously, mature as spermatozoa and leave the lobule. There remain behind only the Sertoli cells—which soon degenerate and disappear—and the residual spermatogonia. The latter are germ cells located only in the apex of the lobule, where it joins the collecting duct system.

[1] The term lobule, introduced by Kingsbury ('02), is used here in preference to the terms "cyst," "capsule," "tubule," etc., employed by various investigators.

Though present, they have remained quiescent during the months preceding the extrusion of the spermatozoa; with the emptying of the lobule, however, or even somewhat previously in many urodeles,

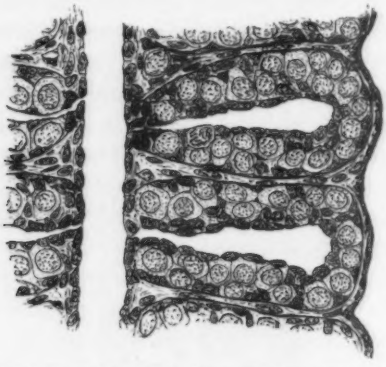

FIG. 2. Longitudinal section of a *Desmognathus* testis, showing a region somewhat posterior to that pictured in Fig. 1. Developing lobules are here well defined and their relation to the collecting duct clearly indicated.

they begin to multiply. By increase in their numbers the emptied lobule is in time completely regenerated; its germ cells again come to maturity and the cycle of degeneration and regeneration is repeated. That a delay in the regeneration of a group of emptied lobules would appreciably modify the form of the testis is self-evident. Such delayed lobule regeneration, as a factor in the formation of multiple testes, will be referred to subsequently.

All the lobules of the urodele testis do not, as a rule, mature and discharge spermatozoa at the same time. Examination of the testis shows the older developmental stages of the germ cells to be localized in its caudal region, while the earlier or younger stages are found more cephalically. In the course of spermatogenesis a wave of developmental change passes from the caudal to the cephalic end of the organ. The lobules of any region, therefore, will contain reproductive elements similarly advanced in development; caudal to any such region older stages are encountered while anterior to it are successively younger stages. Finally, at the anterior end of the testis will be found a slender cord or filament (see Fig. 1) containing only primary spermatogonia.

The caudocephalic movement of the spermatogenetic processes, or, as it has been appropriately termed, the spermatogenetic wave, varies greatly in rate with the species of urodele considered. In *Amblystoma* and *Cryptobranchus* it is comparatively rapid. As a result all regions of the testis tend to be in approximately the same stage of development at any particular time. In other urodeles, such as *Necturus* or *Gyrinophilus*, the rate is considerably slower, and in these several developmental stages will usually be encountered in cephalocaudal succession. Finally, in *Desmognathus* and *Diemyctylus* the rate is extremely slow, the spermatogenetic wave traversing only a part of the length of the testis in any one sexual cycle.[1] At a certain period in the cycle the secondary spermatogonia toward the anterior end of the organ cease to transform into spermatocytes I. A few lobules of these spermatogonia may degenerate entirely or in part, as described by Kingsbury and Hirsh ('12). These degenerated lobules mark a "boundary plane" caudal to which, in the current season, all the reproductive cells are matured as spermatozoa. Anterior to this boundary plane the germ cells are held over until the following season, when the wave resumes its forward movement from the point at which development was checked in the preceding season.

The testis of *Desmognathus*, then, is characterized by a slow movement of the spermatogenetic wave. Consider, now, the action of this factor, in connection with delayed lobule regeneration, as to possible effect on the structure of the testis. The testis of *Plethodon*, which is always of unit structure, may be used for comparison with that of *Desmognathus*, since the two agree in possessing a central[2] longitudinal collecting duct round which the lobules are arranged as are the spokes of a wheel. The two are in contrast, however, both as to the rate of the spermatogenetic wave and the rapidity of lobule regeneration, after extrusion of the spermatozoa. In *Desmognathus* both processes go on slowly; in *Plethodon* both proceed with comparative rapidity, especially the processes of lobule regeneration.

[1] The urodele male, it will be recalled, completes but one sexual cycle annually.

[2] The collecting duct in *Desmognathus* may, however, be more or less superficial towards the ends of the enlargement or lobe.

In developing males of either *Desmognathus* or *Plethodon*, the testis, subsequent to the establishment of the urino-genital connection, consists of but a slender cord of primary spermatogonia much as in Fig. 1, along whose entire length extends the longitudinal collecting duct. So far the testes of the two animals are substantially alike. Neither shows any indication of the establishment of growth centers such as might result in the formation of a many-lobed organ. Preceding the onset of sexual maturity the spermatogonia of the caudal end of the germ cell cord begin division, and eventually fully developed lobules are formed. This development, of course, proceeds caudocephalically in both animals. In *Plethodon*, whose wave rate is the more rapid, lobules are developed and spermatozoa are matured, in the male's first year of sexual maturity, throughout a considerable part of the length of the original germ cell cord. In *Desmognathus*, because of the slower wave rate, spermatogenesis is checked after proceeding but a fraction of the length of the gonad. A boundary plane is established in the manner previously mentioned, and spermatozoa are matured only caudal to its location.

At the time of maturity of the first spermatozoa, then, the testes of the two animals agree in consisting of but one lobe each. That of *Plethodon* is represented diagrammatically by Fig. 24 of Chart I., in which the unshaded area represents the part maturing spermatozoa. The testis of the young *Desmognathus* male is represented by Fig. 1, in which the same scheme of shading is employed. The boundary plane is indicated by the line *b*. The smaller fraction of the testis becoming functional in *Desmognathus* is to be noted.

Following the extrusion of the spermatozoa, in *Plethodon*, lobule regeneration proceeds rapidly. (It begins, indeed, considerably before the spermatozoa leave the lobule.) Though the form of the organ, before all of the more anterior lobules have been emptied and regenerated, will appear as in Fig. 25, prompt regeneration of the lobules during the winter—the spermatozoa being extruded in autumn—brings the testis, by spring, back to the type shown in Fig. 24. The process is repeated in succeeding seasons with no essential variation, save that the spermatogenetic wave, as the male becomes older, travels each year over a greater extent of the anterior portion of the germ cell cord (represented in black in Fig. 24)

in which, during the first season of the male's sexual activity, the germ cells did not develop beyond the secondary spermatogonial stage. In this way the testis is increased in length somewhat from year to year as the animal develops.

In the *Desmognathus* male, however, the extrusion of the spermatozoa in autumn is *not* followed, as in *Plethodon*, by prompt regeneration of the emptied lobules. The few residual spermatogonia located at the apex of each lobule appear to remain quiescent for several months. During the winter the emptied lobules slowly degenerate. The connective tissue cells surrounding them hypertrophy, forming interstitial cells, as described in detail in a previous communication. In the following summer the testis appears as in Fig. 2 of Chart I. The region anterior to the boundary plane *b* in Fig. 1 has developed to form the functional testis, in which the forward movement of the spermatogenetic wave proceeds slowly until again checked at the new boundary plane *b'*. The region containing degenerating lobules and interstitial cells is shown posterior to *b* (cross-hatched). It is to be kept in mind, of course, that the longitudinal collecting duct, surrounded by scattered groups of residual spermatogonia, extends throughout this region. The degenerating lobules and interstitial cells gradually disappear, until finally only the collecting duct and residual spermatogonia remain. In Fig. 2*A* the testis is represented as seen late in the summer following the animal's first year of sexual activity. The extreme caudal part, reduced to a condition similar to that of the anterior cord of spermatogonia, may be referred to as the "caudal germ-cell cord". The functioning region of the testis shown in Fig. 2*A* empties, and the cycle of changes outlined above is repeated. The caudal germ-cell cord, in this way, is increased in length, as shown in Fig. 3. This figure represents the testis of a male as in the summer after the second extrusion of spermatozoa. The positions of the boundary planes of successive seasons are indicated by *b*, *b'*, and *b''*.

Finally, after several months of inactivity, the residual spermatogonia in the most posterior part of the caudal germ-cell cord begin to multiply. Their multiplication leads to the formation of lobules of secondary spermatogonia; these in due season become spermatocytes I. and finally mature as spermatozoa. The testis,

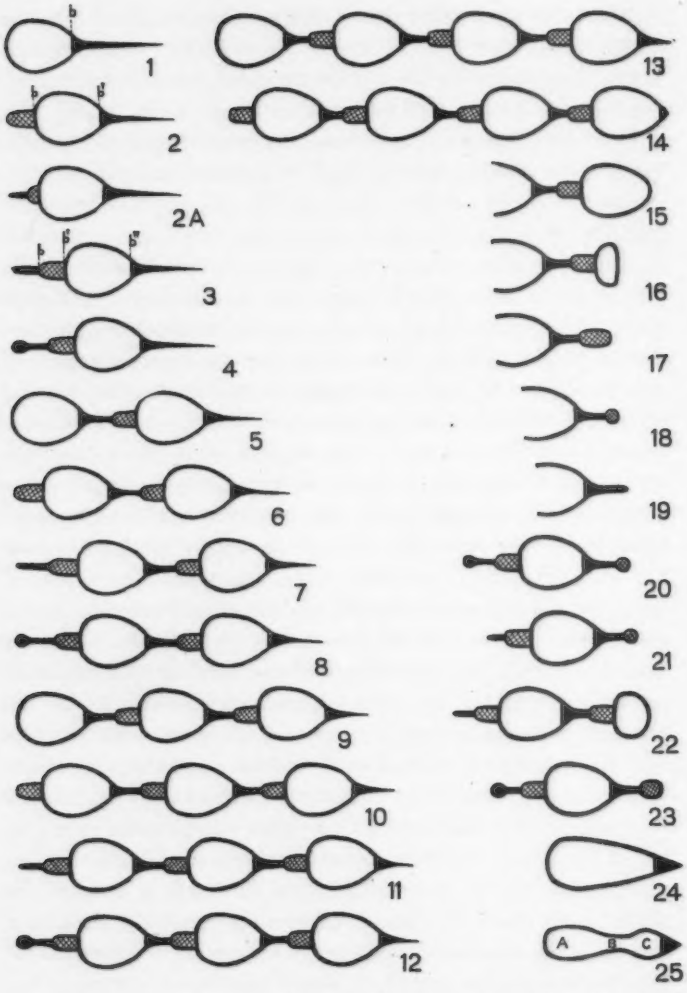

CHART I. FIGS. 1 TO 14. Diagrams illustrating the origin and development of the multiple testis of *Desmognathus*, up to a four-lobed stage. See Tables I. and II. for average lengths of animals having testes of these types. The cephalic end of the testis is at the right in all these figures. Solid black represents regions occupied by primary or secondary spermatogonia. Unshaded areas are those occupied by later stages of the germ cells—spermatocytes I. to mature spermatozoa. Cross-hatched areas are

at the time the lobules first begin regeneration, appears as in Fig. 4. A completely developed second lobe is represented in Fig. 5.

The spermatogenetic wave moves slowly cephalad along the caudal germ-cell cord in the same fashion as it moved along the primary germ-cell cord in the case of the first lobe. Boundary planes are established, in each season, in the same manner; anterior to these the transformation of the spermatogonia into spermatocytes I. ceases. In other words, a second "testis" has been established, caudal to the first and separated from it by the so-called sterile region—a region sterile only in the sense that its germ cells are temporarily inactive. This "testis" or lobe shifts forward on the trail of the first, leaving behind it, when first it empties, a caudal degenerating region such as is represented in Fig. 6. By the disappearance of the degenerating lobules and interstitial cells,

occupied by degenerating lobules and interstitial cells. Fig. 2*A* represents the testis as seen in later summer; all other diagrams are as of testes in June after the boundary plane (*b*, *b'*, etc.) for the season's development of spermatocytes has been established.

FIGS. 15 TO 19. Diagrams of the cephalic portion of the testis of *Desmognathus*, illustrating the disappearance by "running out" of an anterior lobe. Regions of the testis are represented as in the preceding figures. Figs. 15 and 16, testes as in June. Fig. 17, the same testis as in Fig. 15 or 16, but in June one year later. Fig. 18, the same testis later in the summer, prior to the final disappearance of the lobe. Fig. 19, the same testis in September after the last trace of the lobe has disappeared.

FIGS. 20 AND 21. Diagrams of multiple testes of *Desmognathus* approaching a simple type by reduction of the number of lobes.

FIGS. 22 AND 23. Diagrams indicating the differences observed between the testes of an individual. One testis (Fig. 23) is a season ahead of the other in development, as indicated by the earlier disappearance of the anterior lobe and the earlier development of a new lobe caudally.

FIG. 24. Testis of *Plethodon* male for comparison with Fig. 1. Represented as in midsummer. Note the absence of a long anterior germ-cell cord.

FIG. 25. The testis of Fig. 24 as in the fall, some time after extrusion of the spermatozoa had begun. Region *A* contains developing lobules of secondary spermatogonia for the next sexual cycle. Region *B* contains less well-developed lobules, and many degenerating lobules and interstitial cells. In region *C* the lobules still contain spermatozoa, but the lobules of the next cycle are already beginning their development. Black in this and in Fig. 24 indicates the part of the testis not maturing spermatozoa in the first season; *i.e.*, a region containing only primary and secondary spermatogonia. Fig. 25 illustrates the nearest approach to lobation in the testis of *Plethodon*; the rapid development of lobules in regions *B* and *C* soon restores the testis to the type of Fig. 24.

from this region, a caudal germ-cell cord is again established as in Fig. 7; at the posterior end of this cord, eventually, lobule regeneration begins and a third lobe (see Figs. 8 and 9) is produced in exactly the same way as was the second. In this way multiple testes of as many as five lobes may come into being, though this number, I may add, has been found in but one specimen. Likewise, but one specimen with testes of four well-developed lobes has been noted.

The exact time interval that elapses between the first extrusion of spermatozoa and the regeneration of the region they occupied as a caudal second lobe has not been stated in the above description. As lobules emptied in autumn degenerate very slowly, and the interstitial cells marking their former location persist even until the following autumn, the time interval before the emptied region is completely reduced to a simple germ-cell cord is approximately a year. The spermatogonia in this cord, it appears, do not begin their development at any fixed time after the final disappearance of the degenerated lobules, but may remain quiescent for several months longer. Testes similar to those in Figs. 3 and 4 have been taken from specimens killed in June. Judging from the length of the caudal germ-cell cord as compared with that of the functioning testis, the formation of the second lobe could not be considered as having taken place in less than sixteen to eighteen months after the region first matured germ cells. Since the tiny caudal lobes found in June or July will contain only spermatogonia during the current season, they will not ripen spermatozoa until the end of the following summer. In short, the second lobe of a multiple testis may possibly come to full functional maturity only after an interval of three years from the time its territory of the testis first matured sex cells—that is, in the animal's third season of sexual activity. Since after the disappearance of the degenerating lobules and interstitial cells there is no means of distinguishing regions in the caudal germ-cell cord, it is doubtful whether the exact time interval before lobule regeneration begins can be determined. It would appear, indeed, that this time varies in different animals, since in males killed at the same time, with testes having small second lobes developing, these lobes will vary considerably in degree of development; though, doubtless, they are all to be re-

ferred to regions of the testis emptying in the same autumn, in some animals the regeneration of the lobules has been more delayed than in others. It is doubtful, too, whether a caudal lobe, when it develops, occupies exactly the same extent of the germ-cell cord as was occupied by the lobe which preceded it. Caudal lobes of animals killed in September or October may show only a half dozen lobules of mature spermatozoa, or they may be, on the other hand, almost or quite equal in size to the lobes anterior to them. The balance of metabolic processes in the animal doubtless determines the extent of movement of the spermatogenetic wave in any season; hence its cephalic progress in a caudally developing lobe may be checked and the size of the lobe limited quite independently of the exact time that had elapsed since the region was first emptied. The caudal lobe of Fig. 5, therefore, is not necessarily the exact equivalent, in point of territory occupied, of the testis of Fig. 1, but may occupy a greater or less extent of the germ-cell cord.

In each year of sexual activity, as has been stated, the most anterior lobe of the testis is shifted forward by exactly the extent of the region maturing and extruding spermatozoa. It is inevitable that eventually this anterior lobe will reach the cephalic end of the anterior germ-cell cord. By the time this occurs, however, there will be one or more functional lobes established posterior to it. Hence the animal's sexual activity is in no way interfered with by the final disappearance of an anterior lobe.

Such a disappearance or "running out" may be as easily demonstrated as is the caudal addition of new lobes. In Fig. 15 is shown an anterior lobe cephalad of which there is no cord of primary or secondary spermatogonia. This condition marks the first stage in the disappearance of the lobe. In Fig. 16 is represented a lobe somewhat similar to the above, save that it is much shorter; evidently, in the preceding year, only a comparatively small number of lobules of secondary spermatogonia had remained ahead of the boundary plane when it was established, and had been held over for later development. In Fig. 17 a more advanced stage in the running out of a lobe is represented. Here the most anterior lobules of the germ-cell cord have matured and extruded spermatozoa, and are found undergoing degenerative changes, sur-

rounded by sheaths of interstitial cells. Testes of the type shown in Figs. 15 and 16 are frequently encountered in June or July. In another year the testis of Fig. 15 would reach the stage shown in Fig. 17. Then, by the disappearance of the degenerating lobules and interstitial cells during the summer and early fall, all trace of the lobe finally vanishes, as illustrated in Figs. 18 and 19, and only a slender germ-cell cord remains.

The number of lobes that may possibly be formed, then, is limited by the extent of the primary germ-cell cord. This may and doubtless does increase by growth in length of its anterior portion as the animal develops. In any event, it varies considerably in different males, since the anterior lobe may appear as in Fig. 16 or 17 even when only two lobes are present, or it may show no indication of running out in a testis of from three to four lobes. An unusually long germ-cell cord permits the development of numerous lobes; a short cord reduces the number, though in no case has it been apparent that a very large old *Desmognathus* male possessed but a single lobe. Animals have been found, however, with multiple testes (as in Fig. 20) consisting of one large functional lobe, a very small lobe developing caudally, and a trace of an anterior lobe. Similar to these, but one step nearer to a simple testis, is that represented in Fig. 21. In this case no caudal lobe has yet begun its growth, and the anterior lobe is reduced almost to total disappearance. This type of testis in *Desmognathus* is exceptional, only one having been encountered in an examination of over a hundred males.

From the preceding description it is readily seen that a combination of two factors is essential in the production of the multiple testis of *Desmognathus*. These two factors, let me repeat, are the slow movement of the spermatogenetic wave and the delayed regeneration of the emptied lobules. Neither of them alone could give rise to a multiple testis. If, for example, the slow spermatogenetic wave is combined with rapid regeneration of the lobules, the length of the testis is merely increased each year by additions from the anterior germ-cell cord, but remains a unit, as in the *Plethodons*. If, on the other hand, a rapid spermatogenetic wave passed the complete length of the testis in a single season, as in *Cryptobranchus*, but lobule regeneration were then delayed for the

length of time that it is in *Desmognathus*, the animal would, of necessity, pass an interval of one or more years of sexual inactivity before reproduction again became possible. Such a condition, of course, does not exist in any known urodele.

Even in an animal possessing the spermatogenetic pattern requisite for the production of a multiple testis it is further essential that considerable time elapse after sexual maturity before a second testicular lobe is developed, and, following this, still another interval before the appearance of a third. All the evidence from *Desmognathus* is contradictory to Champy's assertion that age is not a factor, and that a male one year of age may possess testes of several lobes. Sexually immature males with more than one lobe are unknown to the writer. Large, heavy-bodied males invariably have multiple testes; these males, however, are always sexually mature, as shown either by the presence of spermatozoa or the occurrence in the testis of degenerating lobules emptied in a previous sexual cycle. Further, these large, heavy-bodied males, when one takes them in large numbers from their natural environment, must also average *older* than slender-bodied males of half to two thirds their body length taken from the same environment. Males of this last class average a smaller number of testicular lobes. *In short, the younger the animal, the smaller the number of lobes that will be found to have developed.*

As previously stated, the writer does not consider that the number of lobes is associated with body size alone, as claimed by Champy. Since, however, body size is to a certain extent correlated with age, an incidental correlation between lobe number and body length may be expected as a result. Examination of *Desmognathus* males shows some sexually mature animals to be only of 6 to 7 cm. total length, while the largest males measure 10 to 12 cm. A graphic representation of the relation of size to the form of the testis is presented in chart II. For graph *A*, stages 1 to 14 in the development of a multiple testis, taken from chart I., are used as abscissæ, and the corresponding average body lengths of the males, as given in Table I., are used as ordinates. It will be noted that up to stage 5 there is a fairly gradual increase in body length, along with the increase in the number of lobes. Animals at this stage—

TABLE I.

LENGTHS OF DESMOGNATHUS MALES FROM STREAMS ON TURKEY HILL, NEAR ITHACA.

Arranged in groups on the basis of the type of testis. These types, listed in first column, correspond in number with the types illustrated in chart I. The one animal with testis of five lobes is not included in this table; since it had suffered the loss of a portion of its tail, its length could only be estimated.

Testis Type No.	Number of Lobes.	Number of Specimens Examined.	Length in Centimeters.		
			Maximum in Group.	Minimum in Group.	Average of Group.
1	1	4	5.8	5.0	5.4 −
2	1 +	11	8.0	5.5	6.7 +
3	1 +	10	9.5	7.3	8.3 −
4	2	18	10.2	8.0	8.6 +
5	2	8	12.2	8.0	9.8 −
6	2 +	16	12.2	8.0	9.8 +
7	2 +	6	11.2	8.0	10.2 −
8	3	5	10.7	10.0	10.3
9	3	4	12.0	9.0	10.6 −
10	3 +	6	10.5	9.7	10.2 −
11	3 +	0	—	—	—
12	4	0	—	—	—
13	4	1	11.0	11.0	11.0
14	4 +	2	12.0	10.5	11.2 +

that is, with testes of two complete lobes—have reached practically their full size, since after this, if we disregard the three animals representing stages 13 and 14, there is but slight upward or downword shifting in the level of the curve. Hence the maximal size may be attained when the testis is at stages 5 to 9, but the number of lobes may continue to increase for some time before checked by the running out of the anterior lobe at the cephalic end of the germ-cell cord. If the correlation were merely one of size rather than of age, one would expect a curve of quite a different type—a curve continuing upward in the same direction as the portion 1 to 5. This portion of the curve, incidentally, shows the correlation of size and lobe number in the sexually mature but growing male; after full size is attained lobe number still increases, and the last half of the curve maintains a rather constant level.

Graph *A* is based upon animals taken largely from streams on Turkey Hill, about three miles from Ithaca. The animals of these

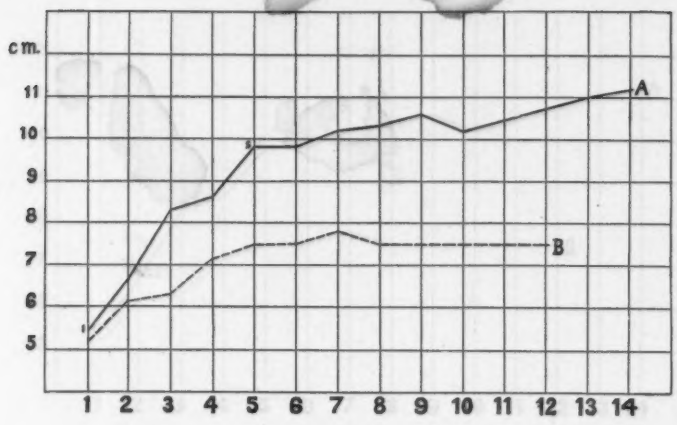

CHART 2. Graph *A* illustrates the relation of testis structure to size in the 93 *Desmognathus* males listed in Table I. The structural types from the first column of this table (see chart 1, Figs. 1 to 14) are here used as abscissæ, and the corresponding average lengths from the last column are used as ordinates. Between stages 5 and 14 an increase in length of but 1.2 cm. is indicated; the number of lobes of the testis increases with age, while the body size, after a certain period, remains relatively fixed. Between stages 1 and 5, on the other hand, there is an increase in length of 4.4 cm. Males mature spermatozoa long before they have attained their maximal size; the processes that operate to produce a multiple testis begin at sexual maturity; hence the development of the first additional lobe is accompanied by the continued increase in body length.

Graph *B*, constructed in the same manner as Graph *A*, shows the relation of testis structure to size in the 24 animals listed in Table II. Between stages 5 and 12 there is here a variation of only 0.3 cm. in average length, as compared with the increase of 1.4 cm. between stages 2 and 5. These animals average much smaller than the animals of the first group, as may be readily seen from the relative heights of the curves at any point. Running out of anterior lobes occurs relatively early and comparatively few lobes are developed; note that stages 9 to 14, represented by 13 males in Table I., have but one representative in Table II.

streams are the largest found in the vicinity of Ithaca, and are uniformly of a lightly colored type. Animals taken from the various gorges nearer Ithaca are ordinarily of smaller size and darker coloration. These have been grouped separately and a second graph (*B*) constructed which agrees in general with the first. For data concerning these animals, see Table II. The animals in this

group, as will be seen, are of much smaller average size and do not develop as many lobes as do the animals of the first group.

The multiple testis being purely the expression of the spermatogenetic wave rate and the time required for lobule regeneration, it follows that conditions modifying these factors will through them modify the form of the testis. Favorable metabolic conditions may increase to some extent the wave rate. The wave will travel farther in a season—*i.e.*, a greater number of lobules of secondary spermatogonia will develop into spermatocytes I. before such development is checked for the season and a boundary plane established. As a result the functional lobe will be of greater length than had the wave rate been slower. Lobes of large vigorous males, it is found, tend to be longer than those of smaller specimens. Greater lobe length, in turn, acts to reduce the number of lobes that may develop from a germ-cell cord of given length. Increase of the spermatogenetic wave rate beyond a certain limit, clearly, would reduce the number of lobes to one, and a multiple testis would not be developed.

Unfavorable metabolic conditions, similarly, may still further reduce the normally slow wave rate of *Desmognathus*. This would

TABLE II.

LENGTHS OF DESMOGNATHUS MALES OF SMALL DARK TYPE, TAKEN FROM GORGES NEAR ITHACA (Largely from Fall Creek Gorge on Cornell University Campus.)

Arranged in groups on the basis of the type of testis. The type numbers in the first column correspond to the type numbers of the diagrams of chart I.

Testis Type No.	Number of Lobes.	Number of Specimens Examined.	Length in Centimeters.		
			Maximum in Group.	Minimum in Group.	Average of Group.
1	1	1	5.2	5.2	5.2
2	1 +	1	6.1	6.1	6.1
3	1 +	5	6.9	5.5	6.3
4	2	5	8.1	6.1	7.1
5	2	2	7.2	7.7	7.5
6	2 +	5	8.2	6.0	7.5
7	2 +	3	8.0	7.5	7.8
8	3	2	8.0	7.0	7.5
9, 10, 11	3 +	0	—	—	—
12	4	1	7.5	7.5	7.5
13, 14	4 and 4 +	0	—	—	—

result in the formation of shorter lobes and permit the establishment of a greater number before a cephalic running out of the first lobe occurred. It seems probable that the presence of several lobes is of itself a factor in reducing wave rate. When a large number of lobes is present, each lobe is shorter, as a rule, than those of males with a smaller number. This acts, of course, in a full-grown animal, to keep the total functioning volume of the testis a constant as new lobes are added.

In the preceding consideration of the effect of wave rate it has been assumed that the time elapsing before lobule regeneration occurs has not been affected. This, however, is probably subject to the influence of metabolic conditions in the same way as is wave rate. We may safely assume, I believe, that lobule regeneration could be somewhat hastened by very favorable conditions or delayed by unfavorable ones. The effect in the first case would be to reduce the length of the interval between lobes, and therefore permit the establishment of a greater number; the effect in the second case would be of the opposite nature.

Favorable metabolic conditions, therefore, would tend to increase the wave rate and through it the size of the lobes, reducing at the same time their possible number; the same conditions, however, would probably cause earlier regeneration, shorten the interval between lobes, and tend thus to permit a greater number. Unfavorable conditions, by slowing the wave rate, shorten the lobes and thus increase the number possible; the same conditions, though, would tend to delay lobule regeneration and the formation of new lobes, increase the interval between lobes, and reduce the number. The net change, so far as the functional volume of the testis is concerned, is small; and excessive increase or reduction of the total output of reproductive cells will not occur, in a fully developed male, except under very unusual conditions.

The writer, therefore, though agreeing that bodily vigor may influence lobe number, insists that it does so only by affecting the length of the primary germ-cell cord, modifying the rate of the spermatogenetic wave, or changing the time necessary for lobule regeneration. In other words, given a spermatogenetic pattern such that multiple testes are possible, the modification of this pattern, by whatever influences it may be brought about, inevitably

modifies to some extent the end result. Hence one may expect a certain variation in lobe number even in animals of exactly the same age.

So, too, on this basis, the occasional differences between the testes of the individual male become understandable. Figures 22 and 23 illustrate the testes from animal number 437, killed on June 26. One testis (Fig. 22) shows a well-developed anterior lobe. In the other (Fig. 23) the disappearance of this lobe is practically complete. Nevertheless the almost microscopic enlargement containing degenerating lobules and interstitial cells shows that a functional lobe occupied this region in the preceding summer. At the caudal end of the testis represented in Fig. 23 a small lobe is developing; in the other (Fig. 22) only a slender caudal germ-cell cord appears. Clearly the one testis is a season in advance of the other, as evidenced both by the earlier disappearance of a lobe anteriorly and the earlier development of a new lobe caudally. Whether in the animal's first year of sexual maturity only one testis had become functional, or whether at some time later the spermatogenetic processes were retarded in one organ, it is impossible to state. Other animals have been found in which the two testes differ only in the size of the anterior lobes, they being of the types shown in Figs. 15 and 16. Still other males show differences only in the stage of development of the posterior lobes; animals are frequently found with one testis of the type shown in Fig. 4, while the other is as well developed as that in Fig. 5. Testes differing as do those of Figs. 5 and 6 have also been removed from a single individual. All the differences so far encountered, however, are but further evidence that the multiple testis is an expression of the spermatogenetic processes. Considering the lobes as structures of a segmental origin, or as divisions of the testis associated merely with the body size, the differences found in a single animal would be difficult of interpretation; realizing the manner in which lobes arise, run their course, and disappear, the occurrence of such differences is even to be expected. Though found in but a small number of the males studied, such differences are by no means rare; and, when found, they are to be looked upon as a normal result of the different modi-

fication in the two organs of those growth, degeneration, and regeneration processes which make a multiple testis possible.

In the writer's opinion, no particular phylogenetic significance attaches to this peculiar type of testis. It occurs in several members of the family Salamandridæ; Champy mentions it in numerous European tritons and salamanders, and I myself have observed it in our two American members of the family. If axolotl, on the other hand, possesses a multiple testis, as Champy states, it differs from its near relative, *Amblystoma punctatum*.[1] Among the Plethodontidæ, too, *Desmognathus* stands alone, no other member of the family, to my knowledge, possessing a multiple testis. Since the plan of spermatogenesis in *Necturus* or *Cryptobranchus*—in fact, in any of the urodeles I have examined—differs from that in *Desmognathus* or the Tritons in no fundamental way, a multiple testis might arise in any of these urodeles if the spermatogenetic processes became sufficiently reduced in rate. In this way, probably, have the species now possessing multiple testes arisen from ancestors with organs of the simple type. Such slowing of the reproductive processes in phylogeny might possibly be interpreted as due to deterioration in the vigor of the stock; the writer sees neither in it nor in the resulting structure of the testis any particular adaptative value.

Summary and Conclusions.

1. Multiple testes of two or more enlargements or lobes separated by intervening non-functional regions are of common occurrence in *Desmognathus* and other urodeles.

2. Such multiple testes are observed only in larger males. The smallest sexually mature males have testes of but a single lobe.

3. A study of the manner in which new lobes appear indicates the following mode of origin:

[1] Spengel says: "Beim Axolotl erscheint er (the testis) als eine breite, dicke, von zahlreichen Unebenheiten besetzte Platte." This description is somewhat more applicable to the testis of *Amblystoma punctatum*. Irregularities, however, are to be observed in the testis of *Amblystoma* only in the spring when the lobules are emptying, and are in no case so extreme as to be at all similar to the lobes of a true multiple testis. It may be questioned whether the "lobes" described by Champy in axolotl are similar in origin to those in *Desmognathus* and the tritons, or whether they are merely such "Unebenheiten" as Spengel mentions. No other investigator has described the testis in axolotl as being of the multiple type.

(*a*) The portion of the testis emptied in autumn is not regenerated immediately, since the residual spermatogonia remaining in the region do not begin division, but remain quiescent for several months.

(*b*) The functional testis in the next season develops anterior to the emptied region, from a part of the original germ-cell cord not developing spermatozoa in the first season.

(*c*) When the emptied lobules degenerate and disappear, the region they occupied remains as a slender strand or cord of residual spermatogonia.

(*d*) Spermatogonia in the caudal end of the cord finally begin division. Lobules of germ cells develop and a caudal "secondary testis" or lobe is formed.

(*e*) The second lobe shifts forward each season in the same manner as does the first. From the germ-cell cord left in its wake a third lobe eventually arises. The process is repeated in the case of other lobes subsequently added.

(*f*) The most anterior lobe finally reaches the cephalic end of the germ-cell cord and disappears. Other lobes follow in due time, but are replaced by new ones originating caudally.

4. New lobes have been found to appear in no other manner than by the process above outlined.

(*a*) Sexually immature males never show numerous growth centers in the germ-cell cord.

(*b*) New lobes do not arise by the promiscuous establishment of secondary growth centers between lobes already present.

(*c*) Small anterior lobes always prove to be disappearing or "running out"; new lobes have not been found to develop in this region.

5. The conditions necessary for the formation of a multiple testis are:

(*a*) A slow forward movement of the spermatogenetic wave.

(*b*) Delayed regeneration of the emptied lobules.

(*c*) The lapse of a time interval following which the long-postponed regeneration brings into existence the new lobe. The unregenerated region between lobes is that commonly termed "sterile."

6. The multiple testis is clearly but a result of the spermatogenetic pattern of the species possessing it.

7. Modifications of the spermatogenetic pattern cause differences in the number or size of the testicular lobes even in animals of the same age. Even in the individual the two organs, from a similar cause, may occasionally be of different type.

8. Neither the multiple testis nor the peculiar combination of factors producing it are regarded as of important phylogenetic or adaptative significance.[1]

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[1] The writer takes pleasure in acknowledging the helpful suggestions and criticisms offered by Dr. B. F. Kingsbury, and the courtesy of the Harvard Museum of Comparative Zoölogy, from whose library the publications of Spengel were obtained.

THE HYDROLYSIS OF HIGHER FATS IN EGG-SECRETION.

OTTO GLASER.

I.

In four recent publications ('15, '18, '21, and '22) Richards, Miss Woodward, and I have dealt with the enzymic properties of *Arbacia* egg-secretion. It was shown, first of all, that radiation affects these secretions as though enzymes were present; next, there was precipitated a body, lipolysin, which, like unmodified exudate, was subsequently shown to accelerate the hydrolysis of ethyl butyrate. Finally, in my last paper ('22), I reported the synthesis of butyric ester.

On the basis of these results, we may assume that lipolysis plays a rôle in the initiation of development. The position is greatly strengthened by the fact that normal eggs may be completely sterilized if their secretions are removed by short exposure to charcoal ('21). Nevertheless the view that initiatory changes are somehow linked with the activities of a lipolytic enzyme requires further evidence. So far the results with ethyl butyrate are the clearest of all. However, if these were due, as they might be, to a relatively specific esterase, the fact, though interesting from other points of view, would be difficult to fit into a theory of fertilization. In order that lipolysis may find a place as part of the mechanism of initiation, it must first be shown that the enzymes present in egg-secretion actually affect the hydrolysis of higher fats.

II.

Preliminary tests were made with olive oil. This was carefully neutralized with dilute NaOH and subsequently extracted with fresh water, and later with ether ('09). The oil was then ready for use.

If egg-secretion influences the hydrolysis of olive oil, the effect, under the proper conditions, might be rendered apparent through

changes localized at the oil-exudate interface. Here important disturbances should take place, although mere volumetric changes are not likely to be reliable. The diffusion of the glycerine set free in hydrolysis would, of course, result in a decrease in the volume of the oil drops. On the other hand, the production of oleic acid with its great affinity for oxygen and its capacity for breaking down into lower fatty acids might easily balance, or even overbalance, the loss resulting from the solubility of the glycerine in egg-water. Conceivably, then, the drops might decrease in volume, increase in volume, or even remain constant.

Nevertheless, if hydrolysis takes place, a lowering of the surface tension at the oil-exudate interface is to be expected, and, regardless of volumetric considerations, should bring about a concentration at the phase-boundary of substances either in solution or in suspension within the oil. If such substances happen to be insoluble in egg-secretion, one may likewise expect their precipitation immediately about the periphery of a drop from which glycerine and possibly the oxidation products of oleic acid, are diffusing outward.

I used two indicators; in some tests Sudan III. was dissolved in the olive oil, in others pulverized charcoal was suspended. A sharp phase-boundary, permitting a clear focus under the microscope, was secured by placing small discs of the oil on the surface of the exudate. The observations were checked by comparisons made on sea-water and distilled, as well as by the use of oil without indicators. Additional controls were carried on with solutions of pancreatin and the commercial Holadin.

With discs of plain olive oil on sea-water, there is some marginal effect. This is indicated by the rather rapid development of a moderately irregular outline. On distilled water the discs maintain their smooth circular form almost perfectly during the first few hours of an experiment. The differences in these two cases are no doubt traceable to the sea salts.

If egg-secretion is used in place of sea-water, the oil discs in the course of an hour take on a form suggestive of a circular saw whose projecting teeth are in a state of active disintegration. Small particles can be seen breaking off in large numbers until the originally sharp outline of the disc becomes quite obscured. Simi-

lar effects were noticed on sea-water, but they develop less rapidly and, within the time limit of these experiments, never equaled the effects gotten with egg-secretion.

With Sudan III., in the early stages of an experiment, peripheral elimination and precipitation of the dye on either sea-water or distilled is questionable. On egg-secretion, however, there is no doubt. The outlines of the discs become exceedingly irregular; there is marked surface concentration of the stain, and very minute particles of it are precipitated densely about the periphery. After 12 hours the oil discs are quite gone. In place of them one finds irregular islands, stained with Sudan III. After 24 hours a rancid odor is noticeable.

With pulverized charcoal exactly comparable results can be gotten, only the surface changes are even more striking. After 24 hours the islands on egg-water can be distinguished macroscopically from their controls, partly because of their greater irregularity, and partly because they have become vesiculated.

If the differences noted are due to the activities of a lipolytic ferment, then solutions known to contain fat-splitting enzymes should reproduce these differences. Attempts were made with two products, the one labeled Pancreatin and of unknown origin, the other a Parke-Davis preparation with the trade name Holadin. Both of these, especially in experiments in which charcoal was used, gave very striking effects and, after 24 hours, the irregular vesiculated islands could be distinguished even macroscopically from their controls.

III.

In these first experiments I made no attempt to control bacterial action. Whatever may be true after six, twelve, or twenty-four hours, the earliest noticeable differences between the oil discs on exudate and on sea-water became apparent so quickly that bacterial digestion seems unlikely. This fact, therefore, warrants a more careful examination of the fat-splitting properties of the secretion.

I now prepared exudate as free as possible from bacterial contamination. The females were thoroughly washed in running fresh water and carefully dried. Only shed eggs were used and these in filtered sea-water. The secretion itself was filtered several

times through Chardin paper and the remaining infection controlled by the addition of chloroform. KCN was not employed because it checks the action of certain lipases.

With exudates of this type I shook up varying proportions of either olive oil or whale oil and at stated intervals attempted to titrate the systems with NaOH N/40 back to the specific turning points of such indicators as phenolphthalein, neutral red, or litmus. On account of the buffer effects and saponifications due to the salts present in both secretion and sea-water, such titrations, even if otherwise reliable, can not disclose the total acidity.

But there are other difficulties. Digests of the type here under consideration are almost proverbially mean. In addition, these particular systems in egg-secretion, in the course of an hour or two, underwent serious physical changes. At the beginning of an experiment, oil and secretion, after shaking, would separate promptly, but after standing for the stated times at 20° C. the separation was much less complete. After four hours even moderate shaking imparted to the systems a stability almost jelly-like.

Under these circumstances, even with the addition of neutral alcohol ('03), titration gave highly variable results, and hence nothing in the nature of a curve showing the course of the reaction can be plotted. However, if we take the most reliable titrations—*i.e.*, those made upon volumes measured before the physical changes previously referred to had taken place—certain very definite comparisons are possible.

Series *A*.....40 c.c. secretion 10 c.c. olive oil	40 c.c. sea-water 10 c.c. olive oil
After 45 hrs.....10 c.c.=2.7 c.c. NaOH *N*/40	10 c.c.=1.9 c.c. NaOH *N*/40
Series *B*.....150 c.c. secretion 25 c.c. olive oil	150 c.c. sea-water 25 c.c. olive oil
After 30 min.....10 c.c.=1.5 c.c. NaOH *N*/40	10 c.c.= .4 c.c. NaOH *N*/40
Series *C*.....90 c.c. secretion 25 c.c. olive oil	90 c.c. sea-water 25 c.c. olive oil
After 30 min.....10 c.c.=1.8 c.c. NaOH *N*/40	10 c.c.=1.3 c.c. NaOH *N*/40

These values indicate the number of c.c. of NaOH N/40 necessary to return the several systems to P_{H_8}, using phenolphthalein as the indicator. The relatively high acidity found in the controls is a disturbing factor, but unavoidable, since oleic acid is never en-

tirely absent in a "neutral" oil; is, moreover, constantly being produced; and, as constantly oxidized to dioxystearic and, very likely, other lower fatty acids. The differences between the digests and the controls, however, remain significant.

IV.

By far the most fruitful observation was made, not on the digests themselves, but on the litmus which in certain cases was used as the indicator. As compared with the controls in sea-water and oil, and in exudate without oil, neutral litmus, when shaken up in mixtures of secretion and either whale oil or olive, instantly becomes pinker. After two hours the pink is distinctly intensified, and after twelve, such digests stand out sharply from their controls.

Within twenty-four hours all color, including the pink, disappears in the oil-secretion digests. If more litmus is now added, it again turns pink, and, in the course of time, fades out completely. The controls, on the other hand, even after forty-eight hours, still appear "neutral," with perhaps only the faintest leaning toward pink.

It is plain that the litmus is not functioning here as a direct indicator of acidity. Very likely the change to pink and the final complete decolorization are the first and last visible steps in a process of reduction. But why, we may ask, the difference between the secretion digests with neutral oil and the controls? If the explanation is to be found in the superior reducing powers of the oil-secretion systems, then these must be producing a substance capable of binding oxygen. Moreover, they must be producing this material at a rate far in excess of the rate in the controls.

Now, if the oils in the presence of secretion are undergoing ordinary lipolytic cleavage, one of the reaction products must be oleic acid, and this, as is well known, absorbs oxygen with great ease. Is it possible, then, to attribute the decolorization of the litmus to the reducing powers of oleic acid? We can only do this if we can first show that oleic acid is present in higher concentration in the digests than in the controls; and, if we succeed in proving this, we shall incidentally also furnish the proof that the only source of oleic acid—the neutral oil—is undergoing accelerated hydrolysis in the presence of egg-secretion.

V.

With this idea in mind, I prepared a special set of tubes, using two volumes of egg-secretion, one volume of either neutral olive or whale oil, and one volume of a neutral litmus solution. The tubes were "chloroformed" as before, then stoppered and shaken.

The secretion used in these tests was made with every precaution possible, and I kept a microscopic check on each tube. In a few instances I could find no organisms whatever either at the beginning or at the end of the period of digestion; in other cases the number was too slight to account for the results, since control tubes, deliberately infected to the point of cloudiness, required forty-eight hours to produce a barely noticeable reduction of the litmus, whereas in the oil-secretion digests the reduction always began instantaneously.

Tubes without chloroform became turbid within forty-eight hours and developed the unmistakable rancid odor. In the tubes with bacterial growth inhibited no rancid odor could be noticed on account of the masking effect of the chloroform. This necessitated some other indicator for the presence of oleic acid.

The test finally chosen was based on a statement of Hammarsten's ('12), and depends on the fact that oleic acid in the presence of cane sugar and sulfuric acid develops a purple color. The usual method was to place a drop of the digest on a slide, to mix with this a drop of saturated solution of cane sugar, and to add from 2 to 5 drops of concentrated sulfuric acid.

Care must be taken not to add the sulfuric too quickly, or to use too much, for if the sugar breaks down suddenly, the carbon set free obscures the reaction in the oleic acid. With these precautions, however, the test is delicate and very reliable. The change in the oleic-acid globules can easily be followed under the microscope. It begins as a slight discoloration, succeeded by pink—rose—and finally a deep purple. At 20° C. the reaction is slow with freshly formed oleic acid, but proceeds more rapidly after the oleic has been exposed for some time to the air.

By this method it was possible to demonstrate the presence of oleic acid in the digests in which chloroform had masked the rancid odor. Moreover, the concentration of oleic acid, as shown by the number, size, distribution, and depth of color of the purple glob-

ules, left no doubt that hydrolysis was proceeding at a faster rate in secretion than in sea-water. In the controls an occasional globule did turn purple, but this was to be expected from our inability to prepare, or, for that matter, even to keep, a fatty oil absolutely neutral.

VI.

It may be taken as demonstrated, then, that *Arbacia* egg-secretion has the power to hydrolyze higher fats. Since neither whale oil, olive oil, nor ethyl butyrate occur in sea-urchin eggs, we must conclude that the lipase present is non-specific.

The question now remains whether the lipolysin first isolated by Miss Woodward also affects the hydrolysis of higher fats.

To determine this, I repeated the experiments described in the preceding section, using, in place of egg-secretion, sea-water solutions of the lipolysin precipitate. The concentrations employed were, roughly, 25 milligrams in 10 c.c. of solvent. To report the results in detail would be a mere reiteration of the experiments with egg-secretion. In every case the evidence for hydrolysis was positive, and leads to the conclusion that the precipitated lipolysin is, or contains, that enzyme which in unmodified egg-water is responsible for the hydrolysis of the higher fats.

AMHERST COLLEGE,
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